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(54) Title: METHOD AND COMPOSITION TO ENHANCE THE EFFICACY OF A VACCINE USING CHEMOKINES

(57) Abstract

The present invention relates to a method to enhance the efficacy of a vaccine in a subject treated with the vaccine comprising administering to the subject in combination with the vaccine a one or more chemokines. The present invention also relates to compositions of vaccines containing chemokines.

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METHOD AND COMPOSITION TO ENHANCE THE EFFICACY OF A VACCINE USING CHEMOKINES

1. CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 60/069,281 filed December 11, 1997.

2. BACKGROUND OF THE INVENTION

The present invention relates to a method to enhance the efficacy of a vaccine by administration of a chemokine, such as macrophage derived chemokine (MDC), in conjunction with the vaccine. The present invention also relates to compositions useful in the method.

2.1. GENERATION OF AN IMMUNE RESPONSE

The introduction of a foreign antigen into an individual elicits an immune response consisting of two major components, the cellular and humoral immune responses, mediated by two functionally distinct populations of lymphocytes known as T and B cells, respectively (see generally Coutinho, 1991, *Immune System, Encyclopedia of Human Biology*, Vol. 4, Ed. Dulbecco, Academic Press, Inc.). A subset of T cells responds to antigen stimulation by producing lymphokines which "help" or activate various other cell types in the immune system.

Another T cell subset is capable of developing into antigen-specific cytotoxic effector cells, which can directly kill antigen-positive target cells. On the other hand, the B cell response is primarily carried out by secretory proteins, antibodies, which directly bind and neutralize antigens.

Helper T cells (TH) can be distinguished from classical cytotoxic T lymphocytes (CTL) and B cells by their cell surface expression of the glycoprotein marker CD4. Although the mechanism by which CD4⁺ TH function has not been fully elucidated, the existence of functionally distinct subsets within the CD4⁺ T cell compartment has been reported (Mosmann and Coffman, 1989, *Ann. Rev. Immunol.*

7:145-173). In the mouse, type 1 helper T cells (TH1) produce interleukin-2 (IL-2) and τ -interferon (τ -IFN) upon activation, while type 2 helper T cells (TH2) produce IL-4 and IL-5. Based on the profile of lymphokine production, TH1 appear to be involved in promoting the activation and proliferation of other T cell subsets including CTL, whereas TH2 specifically regulate B cell proliferation and differentiation, antibody synthesis, and antibody class switching.

A second T cell subpopulation is the classical CTL which express the CD8 surface marker. Unlike most TH, these cells display cytolytic activity upon direct contact with target cells, rather than through the production of lymphokines. *In vivo*, CTL function is particularly important in situations where an antibody response alone is inadequate. Significant experimental evidence indicates that CTL rather than B cells and their antibody products play a principal role in the defense against viral infections and cancer.

A salient feature of both T and B cell responses is their exquisite specificity for the immunizing antigen; however, the mechanisms for antigen recognition differ between these two cell types. B cells recognize antigens by antibodies, either acting as cell surface receptors or as secreted proteins, which bind directly to antigens on a solid surface or in solution, whereas T cells only recognize antigens that have been processed or degraded into small fragments and presented on a solid phase such as the surface of antigen-presenting cells (APC). Additionally, antigenic fragments must be presented to T cells in association with major histocompatibility complex (MHC)-encoded class I or class II molecules. The MHC refers to a cluster of genes that encode proteins with diverse immunological functions. In man, the MHC is known as HLA. Class I gene products are found on all somatic cells, and they were originally discovered as targets of major transplantation rejection responses. Class II gene products are mostly expressed on cells of various hematopoietic lineages, and they are involved in cell-cell interactions in the immune system. Most importantly, MHC-encoded proteins have been shown to function as receptors for processed antigenic fragments on the surface of APC (Bjorkman et al., 1987, *Nature* 329:506-512).

Another level of complexity in the interaction between a T cell and an antigenic fragment is that it occurs only if the MHC molecules involved are the same on the APC and the responding T cells. In other words, a T cell specific for a particular antigenic epitope expresses a receptor having low affinity for self MHC

proteins, which when such MHC proteins on APC are occupied by the epitope, engage the T cell in a stronger interaction leading to antigen-specific T cell activation. The phenomenon of a T cell reacting with a processed antigen only when presented by cells expressing a matching MHC is known as MHC-restriction.

The specificity of T cell immune responses for antigens is a function of the unique receptors expressed by these cells. The T cell receptor (TCR) is structurally homologous to an antibody; it is a heterodimer composed of disulfide-linked glycoproteins. Four TCR polypeptide chains known as α , β , τ , and δ have been identified, although the vast majority of functional T cells express the $\alpha\beta$ heterodimeric TCR. Transfer of α and β genes alone into recipient cells was shown to be both necessary and sufficient to confer antigen specificity and MHC-restriction (Dembic et al., 1986, *Nature* 320:232-238). Thus, the $\alpha\beta$ TCR appears to be responsible for recognizing a combination of antigenic fragment and MHC determinants.

The apparent basis of MHC restriction is that $CD4^+$ T cells express $\alpha\beta$ TCR which recognize antigenic fragments physically associated with MHC class II proteins, while the TCR on $CD8^+$ CTL recognize MHC class I-associated fragments. Thus, $CD4^+$ T cells can recognize only a restricted class of APC that are class II $^+$, whereas $CD8^+$ CTL can interact with virtually any antigen-positive cells, since all cells express class I molecules. $CD4^+$ CTL have been identified, and they are MHC class II restricted, and lyse target cells only if the latter express self-MHC class II determinants associated with specific antigenic fragments. Both CD4 and CD8 molecules also contribute to this interaction by binding to monotypic determinants on the MHC class II and I molecules, respectively.

A second type of TCR composed of $\tau\delta$ heterodimers is expressed by a small percentage of T cells, but the involvement of $\tau\delta$ T cells in antigen-specific recognition is still poorly understood. Some studies have shown that functionally active $\tau\delta$ T cells can be cytolytic in a MHC non-restricted manner.

In summary, the generation of an immune response begins with the sensitization of $CD4^+$ and $CD8^+$ T cell subsets through their interaction with APC that express MHC-class I or class II molecules associated with antigenic fragments. The sensitized or primed $CD4^+$ T cells produce lymphokines that participate in the activation of B cells as well as various T cell subsets. The sensitized $CD8^+$ T cells increase in numbers in response to lymphokines and are capable of destroying any

cells that express the specific antigenic fragments associated with matching MHC-encoded class I molecules. For example, in the course of a viral infection, CTL eradicate virally-infected cells, thereby limiting the progression of virus spread and disease development.

2.2. ANTIGEN PRESENTING CELLS

The presentation of antigens to T cells is carried out by specialized cell populations referred to as antigen presenting cells (APC). Typically, APC include macrophages/monocytes, B cells, and bone marrow derived dendritic cells (DC). APC are capable of internalizing exogenous antigens, cleaving them into smaller fragments in enzyme-rich vesicles, and coupling the fragments to MHC-encoded products for expression on the cell surface (Goldberg and Rock, 1992, *Nature* 357:375-379). Since APC express both MHC-encoded class I and class II glycoproteins, they can present antigenic fragments to both CD4⁺ and CD8⁺ T cells for the initiation of an immune response.

By definition, APC not only can present antigens to T cells with antigen-specific receptors, but can provide all the signals necessary for T cell activation. Such signals are incompletely defined, but probably involve a variety of cell surface molecules as well as cytokines or growth factors. Further, the factors necessary for the activation of naive or unprimed T cells may be different from those required for the re-activation of previously primed memory T cells. The ability of APC to both present antigens and deliver signals for T cell activation is commonly referred to as an accessory cell function. Although monocytes and B cells have been shown to be competent APC, their antigen presenting capacities *in vitro* appear to be limited to the re-activation of previously sensitized T cells. Hence, they are not capable of directly activating functionally naive or unprimed T cell populations.

Although it had been known for a long time that APC process and present antigens to T cells, it was not shown until relatively recently that small antigenic peptides could directly bind to MHC-encoded molecules (Babbit et al., 1985, *Nature* 317:359; Townsend et al., 1986, *Cell* 44:959). However, it is believed that, normally, complex antigens are proteolytically processed into fragments inside the APC, and become physically associated with the MHC-encoded proteins intracellularly prior to

trafficking to the cell surface as complexes. Two distinct pathways for antigen presentation have been proposed (Braciale et al., 1987, *Immunol. Rev.* 98:95-114). It was thought that exogenous antigens were taken up by APC, processed and presented by the exogenous pathway to class II restricted CD4⁺ T cells, while the endogenous pathway processed intracellularly synthesized proteins, such as products of viral genes in virally-infected cells, for association with MHC class I proteins and presentation to CD8⁺ CTL. However, although the two pathways in antigen processing and presentation may still be correct in some respects, the distinction is blurred in light of recent findings that exogenously added antigens may also be presented to class I-restricted CTL (Moore et al., 1988, *Cell* 54:777).

The term "dendritic cells" (DC) refers to a diverse population of morphologically similar cell types found in a variety of lymphoid and non-lymphoid tissues (Steinman, 1991, *Ann. Rev. Immunol.* 9:271-296). These cells include lymphoid DC of the spleen, Langerhans cells of the epidermis, and veiled cells in the blood circulation. Although they are collectively classified as a group based on their morphology, high levels of surface MHC-class II expression, and absence of certain other surface markers expressed on T cells, B cells, monocytes, and natural killer cells, it is presently not known whether they derive from a common precursor or can all function as APC in the same manner. Further, since the vast majority of published reports have utilized DC isolated from the mouse spleen, results from these studies may not necessarily correlate with the function of DC obtained from other tissue types. (Inaba et al., 1997, *J. Exp. Med.* 166:182-194; Hengel et al., 1987, *J. Immunol.* 139:4196-4202; Kaut et al., 1988, *J. Immunol.* 140:3186-3193; Romani et al., 1989, *J. Exp. Med.* 169:1169-1178; Macatonia et al., 1989, *J. Exp. Med.* 169:1255-1264; Inaba et al., 1990, *J. Exp. Med.* 172:631-6640). For example, despite high levels of MHC-class II expression, mouse epidermal Langerhans cells, unlike splenic DC, are not active APC in mixed leucocyte reaction (MLR), unless cultured with granulocyte-macrophage colony stimulating factor (GM-CSF) (Witmer-Pock et al., 1987, *J. Exp. Med.* 166:1484-1498; Heufler et al., 1988, *J. Exp. Med.* 167:700-705). Most human Langerhans cells express the CD1 and CD4 markers, while blood DC do not. Additionally, it has not been established the extent to which the functional characteristics observed with mouse DC are applicable to human DC, especially the DC obtained from non-splenic tissues; in part, due to inherent differences between the

human and murine immune systems.

Recently, a few studies have described the isolation of human DC from the peripheral blood, which involves the use of sheep red blood cells and/or fetal calf serum (Young and Steinman, 1990, *J. Exp. Med.* 171:1315-1332; Freudenthal and Steinman, 1990, *Proc. Natl. Acad. Sci. USA* 87:7698-7702; Macatonia et al., 1989 *Immunol.* 67:285-289; Markowicz and Engleman, 1990, *J. Clin. Invest.* 85:955-961). Engleman et al. described a partial purification procedure of DC from human blood, which does not involve the use of sheep red blood cells and/or fetal calf serum, and showed that the partially purified human DC can, in fact, present exogenous antigens to naive T cells (PCT Publication WO 94/02156 dated February 3, 1994 at page 9, lines 5-32).

Recent studies have indicated that DCs are superior APCs as compared to other APCs such as macrophages and monocytes. First, the potent accessory cell function of DCs provides for an antigen presentation system for virtually any antigenic epitopes which T and B cells are capable of recognizing through their specific receptors. For example, Engleman et al. demonstrate that human DCs can present both complex protein antigens and small peptides to CD4⁺ T cells as well as to as CD8⁺ CTL (PCT Publication WO 94/02156 dated February 3, 1994, Example 7, from page 29, line 10 to page 34, line 16). Engleman et al. also show that the *in vitro* priming effect of DCs does not require the addition of exogenous lymphokines, indicating that DCs produce all of the necessary signals in antigen presentation leading to the activation of T cells (PCT Publication WO 94/02156 dated February 3, 1994, from page 32, line 36 to page 33, line 2). More importantly, DCs can induce a primary CD4⁺ T cell-mediated proliferative response when similarly prepared monocytes can not induce such a response (PCT Publication WO 94/02156 dated February 3, 1994 at page 31, lines 23-30). Similarly, when DCs and monocytes were compared for their ability to present antigens for re-activating secondary T cell response, it was observed that DCs were capable of stimulating a stronger response than monocytes (PCT Publication WO 94/02156 dated February 3, 1994 at page 32, lines 12-16).

2.3. CHEMOKINES

Chemokines, or chemoattractant cytokines, are a subgroup of immune factors

that have been shown to mediate chemotactic and other pro-inflammatory phenomena (see, Schall, 1991, *Cytokine* 3:165-183). Chemokines are small molecules of approximately 70-80 residues in length and can generally be divided into two subgroups, α which have two N-terminal cysteines separated by a single amino acid (CxC) and β which have two adjacent cysteines at the N terminus (CC). RANTES, MIP-1 α and MIP-1 β are members of the β subgroup (reviewed by Horuk, R., 1994, *Trends Pharmacol. Sci.* 15:159-165; Murphy, P.M., 1994, *Annu. Rev. Immunol.* 12:593-633; Baggolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

MCP-1 has been shown to attract monocytes but not neutrophils. MCP-1, MCP-2, and MCP-3 share a pyroglutamate proline NH₂-terminal motif and are structurally closely related to each other and to eotaxin (56% to 71% amino acid sequence identity). MCP-1, MCP-2, and MCP-3 attract monocytes, CD4 $^{+}$ and CD8 $^{+}$ T lymphocytes (Loetscher et al. *FAESB J.* 1994, 8:1055-60), as well as basophil leukocytes. MCP-2, MCP-3, and MCP-4 (but not MCP-1) attracts eosinophil leukocytes. All four MCPs attract activated T lymphocytes, natural killer (NK) cells, and dendritic cells (see Baggolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

Eotaxin acts on eosinophils and is inactive on neutrophils and monocytes, but has weak-to-moderate chemotactic activity toward IL-2-conditioned T lymphocytes (see Baggolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705). Due to its preferential, powerful action on eosinophils and its occurrence in different species, eotaxin is considered to be an important chemokine in the pathophysiology of allergic conditions and asthma (See Baggolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

IP10 is a CXC chemokine attracts human monocytes, T lymphocytes, and NK cells, and Mig attracts tumor-infiltrating T lymphocytes. It has been suggested that IP10 and Mig may also be involved in the regulation of lymphocyte recruitment and the formation of the lymphoid infiltrates observed in autoimmune inflammatory lesions, delayed-type hypersensitivity, some viral infections, and certain tumors (Baggolini et al. *Annu. Rev. Immunol.* 1997, 15:675-705).

SDF-1 (stromal cell-derived factor 1), including SDF-1 and SDF-1 β stimulates the proliferation of B cell progenitors, and attracts mature dendritic cells (Finkel et al. *Immunobiology* 1998, 198:490-500). Synthetic human SDF-1 stimulates monocytes, neutrophils, and peripheral blood lymphocytes, as is indicated by [Ca²⁺]_i changes and chemotaxis. SDF-1 is also a powerful HIV-suppressive factor (See Baggolini et al.

Ann. Rev. Immunol. 1997, 15:675-705).

The amino terminus of the β chemokines RANTES, MCP-1, and MCP-3 has been implicated in the mediation of cell migration and inflammation induced by these chemokines. This involvement is suggested by the observation that the deletion of the amino terminal 8 residues of MCP-1, amino terminal 9 residues of MCP-3, and amino terminal 8 residues of RANTES and the addition of a methionine to the amino terminus of RANTES, antagonize the chemotaxis, calcium mobilization and/or enzyme release stimulated by their native counterparts (Gong et al., 1996, *J. Biol. Chem.* 271:10521-10527; Proudfoot et al., 1996 *J. Biol. Chem.* 271:2599-2603). Additionally, α chemokine-like chemotactic activity has been introduced into MCP-1 via a double mutation of Tyr 28 and Arg 30 to leucine and valine, respectively, indicating that internal regions of this protein also play a role in regulating chemotactic activity (Beall et al., 1992, *J. Biol. Chem.* 267:3455-3459).

The monomeric forms of all chemokines characterized thus far share significant structural homology, although the quaternary structures of α and β groups are distinct. While the monomeric structures of the β and α chemokines are very similar, the dimeric structures of the two groups are completely different. An additional chemokine, lymphotactin, which has only one N terminal cysteine has also been identified and may represent an additional subgroup (γ) of chemokines (Yoshida et al., 1995, *FEBS Lett.* 360:155-159; and Kelner et al., 1994, *Science* 266:1395-1399).

Receptors for chemokines belong to the large family of G-protein coupled, 7 transmembrane domain receptors (GCR's) (See, reviews by Horuk, R., 1994, *Trends Pharmacol. Sci.* 15:159-165; and Murphy, P.M., 1994, *Ann. Rev. Immunol.* 12:593-633). Competition binding and cross-desensitization studies have shown that chemokine receptors exhibit considerable promiscuity in ligand binding. Examples demonstrating the promiscuity among β chemokine receptors include: CCR-1, which binds RANTES and MIP-1 α (Neote et al., 1993, *Cell* 72:415-425), CCR-4, which binds RANTES, MIP-1 α , and MCP-1 (Power et al., 1995, *J. Biol. Chem.* 270:19495-19500), and CCR-5, which binds RANTES, MIP-1 α , and MIP-1 β (Alkhatib et al., 1996, *Science* 272:1955-1958 and Dragic et al., 1996, *Nature* 381:667-674). Erythrocytes possess a receptor (known as the Duffy antigen) which binds both α and β chemokines (Horuk et al., 1994, *J. Biol. Chem.* 269:17730-17733; Neote et al., 1994, *Blood* 84:44-52; and Neote et al., 1993, *J. Biol. Chem.* 268:12247-12249). Thus the sequence and

structural homologies evident among chemokines and their receptors allow some overlap in receptor-ligand interactions.

Godiska et al. identified and described the nucleic acid and amino acid sequences of an additional β chemokine designated macrophage derived chemokine (MDC) (PCT Publication WO 96/40923 dated December 19, 1996, and 1997, *J. Exp. Med.* 185:1595-1604). PCT publication WO 96/40923 further provides materials and methods for the recombinant production of the chemokine, the purified and isolated chemokine protein, and polypeptide analogues thereof. The PCT publication WO 96/40923 does not disclose that the human MDC has chemotactic activity upon DC. While Godiska et al. (1997, *J. Exp. Med.* 185:1595-1604) showed that, in a microchamber migration assay, monocyte-derived DC migrated toward the human MDC, the reference fails to teach that MDC can enhance an immune response to an antigen *in vivo*.

Chang et al. (1997, *J. Biol. Chem.* 272(40):25229-25237), isolated a stimulated T cell chemotactic protein (STCP-1) from an activated macrophage cDNA library. The nucleotide sequence of the STCP-1 is identical to that of the MDC isolated by Godiska et al. (PCT Publication WO 96/40923 dated December 19, 1996, and 1997, *J. Exp. Med.* 185:1595-1604). However, unlike the results observed by Godiska et al. (1997, *J. Exp. Med.* 185:1595-1604), Chang et al. (1997, *J. Biol. Chem.* 272(40):25229-25237) showed that although the STCP-1 acted as a mild chemoattractant for primary activated T lymphocytes and a potent chemoattractant for chronically activated T lymphocytes, the STCP-1 has no chemoattractant activity for monocytes, neutrophils, eosinophils and resting T lymphocytes. Chang et al. further showed that the STCP-1 does not induce Ca^{2+} mobilization in monocytes, dendritic cells, neutrophils, eosinophils, lipopolysaccharide-activated B lymphocytes, and freshly isolated resting T lymphocytes.

2.4. HIV VACCINES

Human immunodeficiency virus (HIV) induces a persistent and progressive infection leading, in the vast majority of cases, to the development of the acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983, *Science* 220:868-870; Gallo et al., 1984, *Science* 224:500-503). The HIV envelope surface glycoproteins are

synthesized as a single 160 kilodalton precursor protein which is cleaved by a cellular protease during viral budding into two glycoproteins, gp41 and gp120. gp41 is a transmembrane glycoprotein and gp120 is an extracellular glycoprotein which remains non-covalently associated with gp41, possibly in a trimeric or multimeric form (Hammerskjold, M. and Rekosh, D., 1989, *Biochem. Biophys. Acta* 989:269-280). The V3 loop of gp120 is the major determinant of sensitivity to chemokine inhibition of infection or replication (Cocchi et al., 1996, *Nature Medicine* 2:1244-1247; and Oravecz et al., 1996, *J. Immunol.* 157:1329-1332).

Although considerable effort is being put into the design of effective therapeutics, currently no curative anti-retroviral drugs against AIDS exist. The HIV-1 envelope proteins (gp160, gp120, gp41) have been shown to be the major antigens for neutralizing anti-HIV antibodies present in AIDS patients (Barin et al., 1985, *Science* 228:1094-1096). Thus far, therefore, these proteins seem to be the most promising candidates to act as antigens for anti-HIV vaccine development. Several groups have begun to use various portions of gp160, gp120, and/or gp41 as immunogenic targets for the host immune system (see, for example, Ivanoff et al., U.S. Pat. No. 5,141,867; Saith et al., PCT publication WO 92/22654; Shafferman, A., PCT publication WO 91/09872; Formoso et al., PCT publication WO 90/07119). Therefore, methods to increase the efficacy of vaccines against HIV, especially vaccines using gp120 as the antigen, are needed.

Additionally a novel vaccine technology, designated genetic vaccination, nucleic acid vaccination or DNA vaccination, has been explored to induce immune responses *in vivo*. Injection of cDNA expression cassettes results in *in vivo* expression of the encoded proteins (Dubensky et al., 1984, *Proc. Natl. Acad. Sci. USA* 81:7529-7533; Raz et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4523; Wolff et al., 1990, *Science* 247:1465-1468), with the concomitant development of specific cellular and humoral immune responses directed against the encoded antigen(s) (Wang et al., 1995, *Hum. Gene Ther.* 6:407-418; Ulmer et al., 1993, *Science* 259:1745-1749; Tang et al., 1992, *Nature* 356:152-154; Michel et al., 1995, *Proc. Natl. Acad. Sci. USA* 92:5307-5311; and Lowrie et al., 1994, *Vaccine* 12:1537-1540). Humoral and cellular responses have been induced to HIV-1 and SIV antigens through various applications of this technology in macaques (Wang et al., 1995, *Virology* 221:102-112; Wang et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:4156-4160; and Boyer et al., 1996, *J. Med.*

Primate. 25:242-250) as well as mice (Wang et al., 1995, *Virology* 221:102-112; Lu et al., 1995, *Virology* 209:147-154; Haynes et al., 1994, *AIDS Res. Hum. Retroviruses* 10 (Suppl. 2):S43-S45; Okuda et al., 1995, *AIDS Res. Hum. Retroviruses* 11:933-943).

Recently, Lekutis et al. (1997, *J. Immunol.* 158:4471-4477), assessed the TH cell response elicited by an HIV-1 gp120 DNA vaccine in rhesus monkeys by isolation of gp120-specific, MHC class II-restricted CD4⁺ T cell lines from the vaccinated animals. Lekutis et al. showed that the isolated cell lines proliferated in response to APC in the presence of recombinant gp120, as well as to APC expressing HIV encoded env protein. Lekutis et al. further showed that these cell lines responded to env by secreting IFN- γ and IFN- α without appreciable IL-4 production. These results demonstrate that the animals exhibited a cellular immune response to the DNA vaccine.

Boyer et al. (1997, *Nature Medicine* 3:625-532), inoculated chimpanzees with an HIV-1 DNA vaccine encoding env, rev, and gag/pol, and found that the immunized animals developed specific cellular and humoral immune responses to these proteins. After challenging the immunized animals with a heterologous chimpanzee titered stock of HIV-1 SF2, Boyer et al. further found, using a Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay, that those animals vaccinated with the DNA vaccine were protected against infection whereas the control animals were not so protected.

Kim et al., (1997 *J. Immunol.* 158:816-826), investigated the role of co-delivery of genes for IL-12 and GM-CSF along with DNA vaccine formulation for HIV-1 antigens env and gag/pol in mice. Kim et al. observed a dramatic increase in specific CTL response from the mice immunized with the HIV-1 DNA vaccine and IL-12. Kim et al. also observed that the co-delivery of IL-12 genes resulted in the reduction of specific antibody response, whereas the codelivery of GM-CSF genes resulted in the enhancement of specific antibody response. Kim et al. further observed that co-delivery of IL-12 gene with a HIV DNA vaccine results in splenomegaly (Kim et al. 1997, *J. Immunol.*, 158:816-826), which has been shown in mice to have toxic effects such as weight reduction or even death (Eng et al., 1995, *J. Exp. Med.* 181:1893; Stevenson et al., 1995, *J. Immunol.* 155:2545; and Orange et al., 1995, *J. Exp. Med.* 181:901).

Notwithstanding the recent developments of the HIV DNA vaccine, there still

exists a need for a method to enhance the efficacy of a vaccine, especially an HIV DNA vaccine. For instance, for efficacious vaccine against HIV-1 one preferably induces both cellular and humoral immune responses to control the infection (Boyer et al., 1997, *Nature Medicine* 3:625-532). The induction of both cellular and humoral immune response by the Berjer et al. method is still quite low because only one of the three immunized chimpanzees developed both cellular and humoral responses. Similarly, although co-delivery of an IL-12 encoding gene with a HIV DNA vaccine, as described in Kim et al. (1997, *J. Immun.* 158:816-826), may have enhanced the cellular immune response, this co-delivery also decreased the humoral response.

Citation of a reference hereinabove shall not be construed as an admission that such reference is prior art to the present invention.

3. SUMMARY OF THE INVENTION. SUMMARY OF THE INVENTION.. SUMMARY OF THE INVENTION

The present invention is based upon the ability of chemokines, such as MDC, Rantes, MIP-1d, MIP-1B, and I-309, to enhance the immune response to an antigen, particularly a vaccine. Accordingly, in a first aspect, the present invention provides a method for enhancing the efficacy of a vaccine, which method comprises administration to a subject of one or more purified chemokines, or biologically active fragments, analogues or derivatives thereof, either concurrently with one or more purified antigens against which an immune response is desired or within a time period either before or after administration of the antigens such that the immune response against the antigens is enhanced.

In a second aspect, the present invention provides a method to enhance the efficacy of a vaccine, which method comprises administration to a subject of a first set of one or more purified nucleic acids comprising one or more nucleotide sequences encoding one or more chemokines, or fragments, derivatives, analogues, and/or truncation isoforms thereof, and a second purified nucleic acid comprising a nucleotide sequence encoding one or more antigens against which an immune response is desired, such that, the one or more chemokine(s) and the antigen(s) are expressed in a coordinated manner upon introduction into a suitable cell. Alternatively, the nucleotide sequences encoding one or more chemokines, or

fragments, derivatives, and/or analogues thereof, and the antigens against which an immune response is desired are present on the same nucleic acid.

In a preferred embodiment, the invention provides a method to enhance the efficacy of an HIV vaccine.

In yet another aspect, the present invention provides a composition comprising an immunogenic amount of one or more purified antigens, an amount of one or more purified chemokines, or a fragments, derivatives, analogues and/or truncation isoforms thereof, effective to enhance the immune response to the antigen. In another aspect, the present invention provides a composition comprising a first set of one or more purified nucleic acids comprising one or more nucleotide sequences encoding one or more chemokines, fragments, derivatives analogues and or truncation isoforms thereof, and a second set of purified nucleic acids comprising one or more nucleotide sequences encoding one or more antigens against which an immune response is desired, such that, the chemokine(s) and the antigen are expressed in a coordinated manner upon introduction into a suitable cell. In a preferred embodiment, the antigen is an HIV antigen. In another preferred embodiment, the chemokine is selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha

chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

4. DESCRIPTION OF FIGURES

Figures 1A and 1B. The nucleotide and amino acid sequences of MDC. 1A depicts the nucleotide sequence of MDC (SEQ ID NO:1), with the coding region indicated by the appearance of the amino acid sequence in the line below; and 1B depicts the amino acid of MDC (SEQ ID NO:2) from GenBank accession no. U83171 (Godiska et al., 1997, *J. Exp. Med.* 185:1595-1604).

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for enhancing the efficacy of a vaccine in a subject comprising administering to the subject one or more purified antigens in conjunction with one or more purified chemokines, or more purified fragments, derivatives or analogues and/or truncation isoforms thereof.

While any chemokine may be employed according to the present invention, the chemokine is preferably selected from the following table:

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC Chemokines	Macrophage-derived chemokine	MDC/STCP-1	u83171; u83239
	Monocyte chemotactic protein 1	MCP-1	x14768
	Monocyte chemotactic protein 2	MCP-2	X99886
	Monocyte chemotactic protein 3	MCP-3	x72308; s57464
	Monocyte chemotactic protein 4	MCP-4	u46767
	activated macrophage specific chemokine 1	AMAC-1	Y13710
	Macrophage inflammatory protein 1 alpha	MIP-1 α	AF043339; X03754; D90144

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC Chemokines (continued)	Macrophage inflammatory protein 1 beta	MIP-1 β	j04130; d90145
	Macrophage inflammatory protein 1 gamma	MIP-1 γ	
	Macrophage inflammatory protein 1 delta	MIP-1 δ	AF031587
	Macrophage inflammatory protein 2 alpha	MIP-2 α	AF043340
	Macrophage inflammatory protein 3 alpha	MIP-3 α	u77035
	Macrophage inflammatory protein 3 beta	MIP-3 β	u77180
	Regulated upon activation, normal T cell expressed and secreted (and its variants)	RANTES	M21211
	I-309		M57502
	EBI1-ligand chemokine	ELC	AB000887
	Pulmonary and activation regulated chemokine	PARC/DC-CK-1/MIP4	AB000221
	Liver and activation-regulated chemokine	LARC	D86955
	Thymus and activation regulated chemokine	TARC	D43767
	Eotaxin (and variants)		D49372; Z69291; Z75669; Z75668
	Human chemokine 1	HCC1; NCC2	Z49270; z49269
	Human chemokine 2	HCC2; NCC3, MIP-5, MIP-1 δ	Z70292
	Human chemokine 3	HCC3	Z70293
	IL-10-inducible chemokine	HCC4	U91746
	liver-expressed chemokine.	LEC; HCC4;NCC4	AB007454
	6Ckine		AF001979
	Exodus 1		u64197
	Exodus 2		U88320
	Exodus 3		U88321
	thymus-expressed chemokine	TECK	U86358
	Secondary Lymphoid tissue chemokine	SLC	AB002409

Chemokine Class	Chemokines	Abbreviations	Accession Number
CC Chemokines (continued)	Lymphocyte and Monocyte chemoattractant; Monotactin	LMC	AF055467
	Activation-induced, chemokine-related molecule	ATAC	x86474
	Myeloid progenitor inhibitory factor-1	MPIF-1; MIP-3 or ckbeta8	u85767
	Myeloid progenitor inhibitory factor-2	MPIF-2	u85768
	Stromal cell-derived factor 1 alpha	SDF-1 α ; PBSF	L36034
CXC chemokines	Stromal cell-derived factor 1 beta	SDF-1 β ; PBSF	L36033
	B-cell-attracting chemokine 1	BLC	AJ002211
	HuMIC		x72755 s60728
	H174		AF002985
	Interferon-stimulated T-cell alpha chemoattractant	I-TAC	AF030514
	Interleukin-8	IL-8	m17017; y00787
	IP-10		X02530
	platelet factor 4	PF4	M20901
	growth-regulated gene-alpha	GRO- α	J03561
	growth-regulated gene-beta	GRO- β	M36820
	growth-regulated gene-gamma	GRO- γ	M36821
	Neutrophil-activating protein 2	NAP-2; CTAP-3	M54995; M38441
C-CHEMOKINES	ENA-78		L37036
	granulocyte chemotactic protein 2	GCP-2	Y08770
C-CHEMOKINES	LYMPHOTACTIN	SCM-1	D63789 D63790
CX β C-CHEMOKINES	Fractalkine/neurotactin		U91835 U84487

The present invention also relates to the use of fragments, analogues and derivatives of the foregoing chemokines, as well as truncation isoforms of such chemokines which are known in the art.

The present invention also relates to therapeutic compositions comprising one or more chemokines, nucleic acids encoding one or more chemokines, derivatives, analogues, and/or truncation isoforms thereof, and nucleic acids encoding the same, that are effective to enhance the immune response of a subject to a vaccine.

In another preferred embodiment of the invention, nucleic acids comprising

nucleotide sequences encoding one or more chemokines or fragments or derivatives, including truncation isoforms, thereof, and encoding one or more antigens against which an immune response is desired, which coding sequences are operatively linked to gene regulatory sequences capable of directing the expression of the one or more chemokines and the one or more antigens upon introduction into a suitable cell, for example, but not limited to, the cell (of a subject), are administered to a subject such that the one or more chemokines, or fragments or derivatives, including truncation isoforms, thereof, and one or more antigens, are expressed in the subject.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections which follow.

5.1. METHODS AND COMPOSITIONS TO ENHANCE THE EFFICACY OF A VACCINE

The present invention provides methods for enhancing the efficacy of a vaccine in a subject, which methods comprise administering to a subject an immunogenic amount of one or more purified antigens against which an immune response is desired in the subject in conjunction with an amount of one or more purified chemokines, or fragments, derivatives, analogues and/or truncation isoforms thereof, effective to enhance the immune response against the antigen. In one aspect, the purified chemokine(s), or fragment(s), derivative(s), analogue(s) and/or truncation isoforms thereof, are administered to the subject concurrently with (e.g., in the same composition with) the purified antigen or antigens against which an immune response is desired. In another, aspect, the purified chemokine(s), or fragment(s), derivative(s), analogue(s) and/or truncation isoforms thereof, are administered either before or after the administration of one or more purified antigens against which immunity is desired in the subject, but is administered within such time that the chemokine(s) enhance the immune response to the one or more antigens. For example, but not by way of limitation, the purified chemokine(s) are administered during the time that the subject mounts an immune response against the administered one or more antigens, or, the purified MDC is administered within, for example, but not limited to, 30 minutes, 1 hour, 5 hours, 10 hours, 1 day, 2 days of (preferably, after) administration of the one or more purified antigens against which immunity is desired.

In a preferred embodiment, the present invention provides compositions comprising an immunogenic amount of one or more purified antigens and an amount of purified MDC, or one or more fragments, derivatives or analogues thereof, effective to enhance the immune response to said antigen and, preferably, the composition further comprises a pharmaceutically acceptable carrier.

A preferred chemokine for use in the methods and compositions of the present invention is any MDC protein, fragment or derivative thereof, that is capable of enhancing the efficacy of a vaccine (for example, but not limited to, as determined by the assays described in Section 5.4, *infra*). In one specific embodiment, the MDC is purified full length MDC, preferably full length MDC having the amino acid sequence of SEQ ID NO: 2 (Figure 1B). In another embodiment, the MDC is a purified protein, the amino acid sequence of which consists of amino acid numbers 2-69 of SEQ ID NO: 2 (Figure 1B). In another specific embodiment, the MDC is a purified protein, the amino acid sequence of which consists of amino acid numbers 3-69 of SEQ ID NO: 2 (Figure 1B). In still another specific embodiment, the MDC is a purified protein, the N-terminal amino acid sequence of which consists of the amino acid sequence Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg-Asp-Tyr-Val-Arg-Tyr-Arg-Leu (portion of SEQ ID NO: 2). In yet another specific embodiment, the MDC is a purified protein, the N-terminal amino acid sequence of which consists of the amino acid sequence Pro-Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg (portion of SEQ ID NO: 2). In yet another specific embodiment, the MDC is a purified derivative of a protein, the N-terminal amino acid sequence of which protein consists of the amino acid sequence Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg-Asp-Tyr-Val-Arg-Tyr-Arg-Leu (SEQ ID NO:2), which derivative has activity to enhance the efficacy of the vaccine. In yet another specific embodiment, the MDC is a purified derivative of a protein, the N-terminal amino acid sequence of which protein consists of the amino acid sequence Pro-Tyr-Gly-Ala-Asn-Met-Glu-Asp-Ser-Val-Cys-Cys-Arg (SEQ ID NO:2), which derivative has activity to enhance the efficacy of the vaccine.

In yet another specific embodiment, the chemokine is a purified derivative of the protein, which derivative has one or more insertions of or substitutions with one or more non-classical amino acids relative to a corresponding wildtype chemokine, which derivative will enhance the efficacy of the vaccine. In yet another specific

embodiment, the chemokine is a purified derivative of the protein that has only one or more conservative substitutions in sequence relative a corresponding wildtype chemokine, which derivative will enhance the efficacy of the vaccine. The chemokines useful in the present invention may be derived from any suitable source and obtained by any method known in the art, for example but not limited to the methods described in Section 5.2 infra.

Preferably, the chemokine(s) are of the same species as the subject to which the vaccine is administered. In a preferred embodiment, one or more human chemokines are administered to a human subject, e.g., human MDC is administered to a human subject, alone or in combination with another chemokine.

The present invention also provides a method to enhance the efficacy of a vaccine in a subject, which method comprises administering to a subject a purified first nucleic acid comprising a nucleotide sequence encoding an antigen against which an immune response is desired in a subject and a purified second nucleic acid comprising a nucleotide sequence encoding one or more chemokines, or fragment(s), derivative(s) or analogue(s) thereof, where the expression of the encoded antigen(s) and chemokine(s), or fragment(s), derivative(s) or analogue(s) thereof, are under control of one or more appropriate gene regulatory elements (which regulatory elements can be any regulatory element known in the art, for example, but not limited to, those regulatory elements described in Section 5.2 supra), such that, upon introduction of said first and second nucleic acids into a suitable cell (e.g., a cell of the subject), the antigen and chemokine(s), or fragment(s), derivative(s) or analogue(s) thereof, are coordinately expressed, *i.e.*, are expressed either at the same time or within an appropriate time period (*i.e.*, sufficient for the chemokine(s) to enhance the immune response against the antigen relative to a corresponding immune response in the absence of the chemokine) and the antigen(s) are expressed in an immunogenic amount and the chemokine(s), or fragment(s), derivative(s) or analogue(s) thereof, are expressed in an amount sufficient to enhance the immune response against the antigen(s). In a specific embodiment, the nucleotide sequences encoding the chemokine(s) and the antigen are present on separate nucleic acids. In another embodiment, the nucleotide sequences encoding the chemokine(s) and the antigen(s) are present on the same nucleic acid.

The present invention also provides compositions to enhance the

efficacy of a vaccine in a subject, which compositions comprise a purified first nucleic acid comprising a nucleotide sequence encoding one or more antigen(s) and a purified second nucleic acid comprising a nucleotide sequence encoding one or more chemokines, or fragments or derivatives, including truncation isoforms, thereof, wherein the nucleotide sequences encoding the antigens and the chemokine(s) are operably linked to one or more gene regulatory elements such that, upon introduction of said first and second nucleic acids into a suitable cell (e.g., a cell of the subject), the antigen(s) and chemokine(s) are expressed in a coordinated manner and the antigen(s) are expressed in an immunogenic amount and the chemokine(s) are expressed in an amount effective to enhance the immune response against the antigen, relative to a corresponding immune response in the absence of such chemokine(s).

The present invention also provides compositions to enhance the efficacy of a vaccine in a subject, which compositions comprise a purified first set of one or more purified nucleic acids comprising one or more nucleotide sequences encoding one or more antigens and a purified second set of one or more purified nucleic acids comprising a nucleotide sequence encoding one or more chemokines, or fragments, analogues, derivatives, (including truncation isoforms) thereof, wherein the nucleotide sequence(s) encoding the antigen(s) and the chemokine(s) are operably linked to one or more gene regulatory elements such that, upon introduction of said first and second sets of nucleic acids into a suitable cell (e.g., a cell of the subject), the antigen(s) and chemokine(s) are expressed in a coordinated manner and the antigen(s) are expressed in an immunogenic amount and the chemokine(s) are expressed in an amount effective to enhance the immune response against the antigen, relative to a corresponding immune response in the absence of such chemokine(s).

The present invention also provides compositions to enhance the efficacy of a vaccine in a subject, which compositions comprise a purified nucleic acid comprising a first set of one or more nucleotide sequences encoding one or more antigens and a second set of one or more nucleotide sequence encoding one or more chemokines, or fragments, derivatives, or analogues thereof (including truncation isoforms), wherein the first and second sets of nucleotide sequences are operably linked to one or more gene regulatory elements such that, upon introduction into a suitable cell, the antigen(s) and the chemokine(s) are expressed in a coordinated manner and the antigen(s) are expressed in an immunogenic amount and the chemokine(s) are

expressed in an amount effective to enhance the immune response against the antigen(s).

Any nucleic acid comprising a nucleotide sequence encoding one or more chemokine proteins, or fragments or derivatives, thereof (including truncation isoforms), that are capable of enhancing the immune response to the antigen (for example, but not limited to, as determined by any of the assays described in Section 5.2., *infra*) can be used in the methods and compositions of the present invention.

In a preferred embodiment, the nucleotide sequence encodes MDC. In another embodiment, the MDC-encoding nucleotide consists of the nucleotide sequence of SEQ ID NO:1 (Figure 1A). In another specific embodiment, the method or composition of the invention uses a nucleic acid encoding an MDC derivative having deletional, insertional or substitutional mutations and combination thereof, which derivative has activity to enhance the immune response against an antigen in a subject.

Such compositions of nucleic acids encoding an antigen are often referred to as DNA vaccines.

Such DNA vaccines are produced by any method known in the art for constructing an expression plasmid vector containing the nucleotide sequences of the antigen(s) and/or chemokine(s) to be expressed which vector is suitable for expression of the encoded proteins in the subject or in cells recombinant for the expression vector, which cells are to be provided to the subject. Such expression vectors may contain various promoters, terminators and polyadenylation coding regions to control the expression of the encoded protein.

The DNA vaccine can be administered by any method known in the art for administration of DNA. The DNA vaccine may be delivered either directly, in which case the subject is directly exposed to the DNA vaccine such that the DNA enters and is expressed in cells of the subject, or indirectly, in which case, the DNA vaccine is first introduced into suitable cells by any method known in the art *in vitro*, then the cells containing the DNA vaccine are transplanted into the subject.

In a specific embodiment, the DNA vaccine is directly administered *in vivo*, where it is expressed to produce the encoded antigens and chemokine(s). This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or

other viral vector (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biostatic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In a preferred embodiment, the nucleic acid of a DNA vaccine is injected into the muscle of the subject to be immunized.

Another approach is to introduce the nucleic acid of the DNA vaccine into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign nucleic acid into cells (see e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92 (1985)) and may be used in accordance with the present invention. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene.

Cells into which a DNA vaccine can be introduced for purposes of immunization encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

The resulting recombinant cells can be delivered to a subject by various

methods known in the art. In a preferred embodiment, the recombinant cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The cells can also be encapsulated in a suitable vehicle and then implanted in the subject (see, e.g., Dionne et al. PCT Publication WO 92/19195, dated November 12, 1992). The amount of cells envisioned for use depends on the desired effect, subject state, etc., and can be determined by one skilled in the art.

By way of example, and not by way of limitation a DNA vaccine may be generated as described by Lekutis et al. for an HIV DNA vaccine (1997, *J. Immunol.* 158:4471-4477). Briefly, an expression vector is constructed with the promoter, enhancer and intron A of human cytomegalovirus (CMV) and the termination and polyadenylation sequences of bovine growth hormone in a plasmid backbone. Additionally, the nucleotide sequence for signal sequence of tissue plasminogen activator is either substituted for the signal sequence of the antigen, if the antigen has a signal sequence or is added onto the amino-terminus of the antigen, thereby eliminating the dependence on viral proteins for expression (e.g., in the case of gp120 expression, rev and env proteins are required unless the HIV-1 signal sequence is so substituted). The resulting formulation is then injected intra-muscularly.

Further examples of DNA vaccines are set forth in Boyer et al. (1996, *J. Med. Primatol.*, 25:242-250), which describes the construction of a plasmid encoding the HIV-1 gp160 envelope glycoprotein as well as the rev-tax region cloned into pMAMneoBlue vector (Clonetech, Inc., Palo Alto, CA), and a vector encoding the envelope glycoprotein and rev from HIV-1 strain MN under the control of the CMV promoter. Another vector which can be used in the present invention is as described in Boyer et al. (1997, *Nature Medicine* 3:526-532) and contains expression cassettes encoding the envelope and Rev proteins of HIV-1 strain MN, and encoding the Gag/Pol proteins of HIV-1 strain IIIB.

For the practice of the present invention, the nucleotide sequence for the one or more chemokines, or fragments, derivatives, or analogues thereof, can either be incorporated into the same expression vector containing the nucleotide sequence encoding the antigen in such a manner that the chemokine(s) are expressed. Alternatively, the nucleotide sequence encoding the chemokine(s), or fragment(s),

derivative(s) or analogue(s) thereof, can be cloned into a separate expression vector (e.g., as described above for the expression vector containing the sequences coding for antigen) and the expression vector that expresses the antigen(s) mixed with the expression vector that expresses the chemokine(s). The mixture of the two expression vectors can then be administered to the subject.

The methods and compositions of the present invention may be used as a vaccine in a subject in which immunity for the antigen(s) is desired. Such antigens can be any antigen known in the art to be useful in a vaccine formulation. The methods and compositions of the present invention can be used to enhance the efficacy of any vaccine known in the art. The vaccine of the present invention may be used to enhance an immune response to infectious agents and diseased or abnormal cells, such as but not limited to bacteria, parasites, fungi, viruses, tumors and cancers. The compositions of the invention may be used to either treat or prevent a disease or disorder amenable to treatment or prevention by generating an immune response to the antigen provided in the composition. In one preferred embodiment, the antigen(s) are proteins, fragments or derivatives, including truncation isoforms, thereof, encoded by any genes of the HIV genome including the *env*, *gag*, *pol*, *nef*, *vif*, *rev*, and *tat* genes. In a more preferred embodiment, the antigen is an HIV-associated gp120 protein.

The methods and compositions of the present invention may be used to elicit a humoral and/or a cell-mediated response against the antigen(s) of the vaccine in a subject. In one specific embodiment, the methods and compositions elicit a humoral response against the administered antigen in a subject. In another specific embodiment, the methods and compositions elicit a cell-mediated response against the administered antigen in a subject. In a preferred embodiment, the methods and compositions elicit both a humoral and a cell-mediated response.

The subjects to which the present invention is applicable may be any mammalian or vertebrate species, which include, but are not limited to, cows, horses, sheep, pigs, fowl (e.g., chickens), goats, cats, dogs, hamsters, mice and rats, monkeys, rabbits, chimpanzees, and humans. In a preferred embodiment, the subject is a human. The compositions and methods of the invention can be used to either prevent a disease or disorder, or to treat a particular disease or disorder, where an immune response against a particular antigen or antigens is effective to treat or prevent the

disease or disorder. Such diseases and disorders include, but are not limited to, viral infections, such as HIV, CMV; hepatitis, herpes virus; measles; etc, bacterial infections, fungal and parasitic infections, cancers, and any other disease or disorder amenable to treatment or prevention by eliciting an immune response against a particular antigen or antigens. In another preferred embodiment, the subject is infected or at risk of being infected with HIV virus.

In another preferred embodiment the invention provides methods and compositions to enhance the efficacy of an HIV vaccine, such a vaccine can be administered to either prevent or treat HIV.

5.2. CHEMOKINE GENES AND PROTEINS

Chemokine proteins and nucleic acids can be obtained by any method known in the art. Chemokine nucleotide and amino acid sequences are available in public databases such as Genbank and are also published in various references known to those of skill in the art. The gene bank accession numbers for the preferred chemokines of the present invention are provided in Table I, in Section 5 above. The ensuing discussion uses MDC by way of example, but applies equally to other chemokines as well.

The MDC nucleotide and amino acid sequences for, *inter alia*, human, are available in the public databases (e.g. Genbank accession No. U83171) also published in Godiska et al., 1997, *J. Exp. Med.* 185:1595-1604. The nucleotide sequence and the amino acid sequence for the human MDC are provided in Figures 1A and B (SEQ ID NOS:1 and 2, respectively).

Chemokines used herein include, but are not limited to, chemokines from mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, and human. In one preferred embodiment, the chemokine is of human origin.

Any vertebrate cell potentially can serve as the nucleic acid source for the isolation of chemokine nucleic acids. The nucleic acid sequences encoding the chemokine(s) can be isolated from vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, as well as additional primate sources, etc. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a

DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from cDNA, cDNA is generated from totally cellular RNA or mRNA by methods that are well known in the art. The gene may also be obtained from genomic DNA, where DNA fragments are generated (e.g. using restriction enzymes or by mechanical shearing), some of which will encode the desired gene. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing all or a portion of the chemokine gene may be accomplished in a number of ways.

A preferred method for isolating a chemokine gene is by the polymerase chain reaction (PCR), which can be used to amplify the desired chemokine sequence in a genomic or cDNA library or from genomic DNA or cDNA that has not been incorporated into a library. Oligonucleotide primers which would hybridize to chemokine sequences can be used as primers in PCR.

Additionally, a portion of the chemokine (of any species) gene or its specific RNA, or a fragment thereof, can be purified (or an oligonucleotide synthesized) and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, *Science* 196:180; Grunstein, M. And Hogness, D., 1975, *Proc. Natl. Acad. Sci. U.S.A.* 72:3961). Those DNA fragments with substantial homology to the probe will hybridize. Chemokine nucleic acids can be also identified and isolated by expression cloning using, for example, anti-chemokine antibodies for selection.

Alternatives to obtaining the chemokine DNA by cloning or amplification

include, but are not limited to, chemically synthesizing the gene sequence itself from the known chemokine sequence or making cDNA to the mRNA which encodes the chemokine protein. Other methods are possible and within the scope of the invention. Once a clone has been obtained, its identity can be confirmed by nucleic acid sequencing (by any method well known in the art) and comparison to known chemokine sequences. DNA sequence analysis can be performed by any techniques known in the art, including but not limited to the method of Maxam and Gilbert (1980, *Meth. Enzymol.* 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, *Proc. Natl. Acad. Sci. U.S.A.* 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), use of an automated DNA sequenator (e.g., Applied Biosystems, Foster City, CA) or the method described in PCT Publication WO 97/ 15690.

Nucleic acids which are hybridizable to a chemokine nucleic acid, or to a nucleic acid encoding a chemokine derivative can be isolated, by nucleic acid hybridization under conditions of low, high, or moderate stringency (see also Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA* 78:6789-6792). For example, the nucleic acid of SEQ ID No: 1 is hybridizable to an MDC nucleic acid.

Chemokine proteins and derivatives, analogs and fragments of chemokine proteins can be obtained by any method known in the art, including but not limited to recombinant expression methods, purification from natural sources, and chemical synthesis.

For example, chemokines can be obtained by recombinant protein expression techniques. For recombinant expression, the chemokine gene or portion thereof is inserted into an appropriate cloning vector for expression in a particular host cell. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site

desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and chemokine gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated chemokine gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequence coding for a chemokine protein or a functionally active analog or fragment or other derivative thereof, can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native chemokine gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein

coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a chemokine protein or peptide fragment may be regulated by a second nucleic acid sequence so that the chemokine protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a chemokine protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control chemokine expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff, et al., 1978, *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25); see also "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, *Cell* 38:639-646; Ornitz et al., 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, *Cell* 38:647-658; Adames et al., 1985, *Nature* 318:533-538; Alexander et al., 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, *Cell* 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer et al., 1987, *Science* 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region

which is active in myeloid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378).

For example, a vector can be used that comprises a promoter operably linked to an chemokine-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (e.g., an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by subcloning a chemokine coding sequence into the *Eco*RI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, *Gene* 7:31-40). This allows for the expression of the chemokine protein product from the subclone in the correct reading frame.

Expression vectors containing chemokine gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a chemokine gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted chemokine gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a chemokine gene in the vector. For example, if the chemokine gene is inserted within the marker gene sequence of the vector, recombinants containing the chemokine insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the chemokine protein in *in vitro* assay systems, e.g., binding with anti-chemokine antibody or the chemokine's receptor.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host

system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous protein. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

In other specific embodiments, the chemokine protein(s), fragment(s), analogue(s), or derivative(s) may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. In a specific embodiment, a chimeric protein containing all or a portion of the chemokine is joined via a peptide bond to all or a portion of an antigen against which immunity is desired.

Both cDNA and genomic sequences can be cloned and expressed.

The chemokine protein(s) may also be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column

chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.5). Alternatively, the protein can be synthesized by standard chemical methods known in the art (e.g., see Hunkapiller, M., et al., 1984, *Nature* 310:105-111). The chemokine-encoding nucleic acid sequence(s) can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions. Any technique for mutagenesis known in the art can be used, including, but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), use of TAB linkers (Pharmacia), mutation-containing PCR primers, etc.

The experimentation involved in mutagenesis consists primarily of site-directed mutagenesis followed by phenotypic testing of the altered gene product. Some of the more commonly employed site-directed mutagenesis protocols take advantage of vectors that can provide single stranded as well as double stranded DNA, as needed. Generally, the mutagenesis protocol with such vectors is as follows. A mutagenic primer, i.e., a primer complementary to the sequence to be changed, but consisting of one or a small number of altered, added, or deleted bases, is synthesized. The primer is extended *in vitro* by a DNA polymerase and, after some additional manipulations, the now double-stranded DNA is transfected into bacterial cells. Next, by a variety of methods, the desired mutated DNA is identified, and the desired protein is purified from clones containing the mutated sequence. For longer sequences, additional cloning steps are often required because long inserts (longer than 2 kilobases) are unstable in those vectors. Protocols are known to those skilled in the art and kits for site-directed mutagenesis are widely available from biotechnology supply companies, for example from Amersham Life Science, Inc. (Arlington Heights, IL) and Stratagene Cloning Systems (La Jolla, CA).

In other specific embodiments, the chemokine derivative(s) or analogue(s) may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analogue, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art.

In addition, chemokine proteins, derivatives (including fragments and chimeric proteins), and analogues can be chemically synthesized. See, e.g.; Clark-Lewis et al.; 1991, *Biochem.* 30:3128-3135 and Merrifield, 1963, *J. Amer. Chem. Soc.* 85:2149-2156. For example, chemokines, derivatives and analogues can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 50-60). Chemokines, derivatives and analogues that are proteins can also be synthesized by use of a peptide synthesizer. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, *Proteins, Structures and Molecular Principles*, W.H. Freeman and Co., N.Y., pp. 34-49).

The chemokine proteins, derivatives, or analogues of the invention may be synthesized in their entirety by the sequential addition of amino acid residues or alternatively as fragment subcomponents which may be combined using techniques well known in the art, such as, for example, fragment condensation (Shin et al., 1992, *Biosci. Biotech. Biochem.* 56:404-408; Nyfeler et al., 1992, *Peptides*, Proc. 12th Amer. Pep. Soc., Smith and Rivier (eds), Leiden, pp 661-663); and Nokihara et al., 1990, *Protein Research Foundation*, Yanaihara (ed), Osaka, pp 315-320).

In a less preferred embodiment, chemokine derivatives can be obtained by proteolysis of the protein followed by purification using standard methods such as those described above (e.g., immunoaffinity purification).

In another alternate embodiment, native chemokine proteins can be purified from natural sources, by standard methods such as those described above (e.g., immunoaffinity purification).

5.3. COMPOSITION FORMULATIONS AND METHODS OF ADMINISTRATION

The composition formulations of the invention comprise an effective immunizing amount of an immunologically active ingredient, i.e., one or more antigens, and an amount of one or more chemokine(s), or fragment(s) or derivative thereof, effective to enhance the immune response against the antigen in a subject, and a pharmaceutically acceptable carrier or excipient. In a specific embodiment, the

chemokines are selected from the group consisting of Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

Pharmaceutically acceptable carriers or excipients are well known in the art and include but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, sterile isotonic aqueous buffer, and combinations thereof. One example of such an acceptable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc. The carrier is preferably sterile. The formulation should suit the mode of administration.

In addition, if desired, the vaccine or composition preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine or composition. Suitable adjuvants may include, but are not limited to: mineral gels,

e.g., aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols; polyanions; peptides; oil emulsions; alum; MDP, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine. The effectiveness of an adjuvant may be determined by comparing the induction of antibodies directed against a MDC-containing composition in the presence and in the absence of various adjuvants.

In instances where the recombinant antigen is a hapten, i.e., a molecule that is antigenic in that it can react selectively with cognate antibodies, but not immunogenic in that it cannot elicit an immune response, the hapten may be covalently bound to a carrier or immunogenic molecule; for instance, a large protein such as serum albumin will confer immunogenicity to the hapten coupled to it. The hapten-carrier may be formulated for use as a vaccine.

The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

The chemokine(s), or fragment(s) or derivative(s) thereof, and/or the antigen(s) may be formulated into the composition as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with free carboxyl groups may also be derived from inorganic bases, such as, for example, sodium potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

The vaccines of the invention may be multivalent or univalent. Multivalent vaccines are made from recombinant viruses that direct the expression of more than one antigen.

An effective dose (immunizing amount) is that amount sufficient to produce an immune response to the antigen(s) in the host to which the vaccine preparation is administered. The precise dose of the composition to be employed in the formulation will depend on the route of administration, and the nature of the subject to be

immunized, and should be decided by the practitioner according to standard clinical techniques. Effective doses of the vaccines or compositions of the present invention may also be extrapolated from dose-response curves derived from animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers comprising one or more of the ingredients of the composition formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, a lyophilized immunologically active ingredient and one or more chemokine polypeptide(s) of the invention are provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (e.g., 0.005% brilliant green).

Many methods may be used to introduce the composition formulations of the invention; these include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, e.g., using a bifurcated needle).

The DNA vaccines of the invention can be administered by any method known in the art for delivery of DNA to subject (for example, as described in Section 5.3 *supra*)

5.4. DETERMINATION OF COMPOSITION EFFICACY

The activity of one or more chemokines, or a fragment, derivative or analogue thereof, to enhance immune response to an antigen can be determined by monitoring the immune response in test animals following immunization with a composition containing the chemokine(s) and an antigen and comparing the response to that following immunization with the antigen in the absence of the chemokine(s). Generation of a humoral (antibody) response and/or cell-mediated immunity, may be taken as an indication of an immune response. Test animals may include mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, etc., and eventually human subjects. Assays for humoral and cell-mediated immunity are well known in the art.

Methods of introducing the composition may include oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal or any other standard routes of immunization. The immune response of the test subjects can be analyzed by various approaches well known in the art, such as but not limited to: testing the reactivity of the resultant immune serum to the antigen of the chemokine-containing vaccine, as assayed by known techniques, e.g., immunosorbant assay (ELISA), immunoblots, radioimmunoprecipitations, etc.

As one example of suitable animal testing, a composition of the present invention may be tested in mice for the ability to enhance an antibody response to an antigen (using for example, but not limited to, the method as described in Section 6, *infra*) and the delayed-type hypersensitivity (DTH) response (also described in Section 6 *infra*), measured by an increase in footpad swelling after inoculation in the footpad of the test animal, as compared to the measurements in animals administered the antigen in a composition not containing chemokine. For example, as test animals BALB/c mice may be used. The test group each receives an inoculation with fixed amount of antigen and varying amount of one or more chemokines. The control group receives an inoculation of comparable amount of antigen alone.

Serum samples may be drawn from the mice after the final inoculation (for example every one or two weeks after inoculation), and serum is analyzed for antibodies against the antigen using known methods in the art, e.g., using an ELISA. DTH responses to the antigen may be measured after the final inoculation (e.g. within 1-7 days). An increase in the serum titer of antibodies recognizing the antigen and/or

an increase in footpad swelling in the animals receiving the antigen-compositions containing the chemokine(s) as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing the chemokine(s), indicates that the chemokine(s) enhance the immune response to antigen. An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad swelling in the animals receiving the antigen-compositions containing the chemokines as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing chemokine(s), indicates that the chemokine(s) enhances the immune response to antigen. An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad swelling in the animals receiving the antigen-compositions containing MDC as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing MDC, indicates that the MDC enhances the immune response to antigen. An increase in the serum titer of antibodies recognizing the antigen and/or an increase in footpad swelling in the animals receiving the antigen-compositions containing MDC as compared to the serum titer of antibodies against the antigen and/or the footpad swelling in the animals receiving the antigen composition not containing MDC, indicates that the MDC enhances the immune response to antigen.

6. EXAMPLE: IMMUNIZATION WITH MDC-CONTAINING COMPOSITION

The following experiment illustrates the evaluation of whether MDC will act as an adjuvant for a protein antigen and enhance the efficacy of a vaccine. However, it will be appreciated that the description applies equally to other chemokines and combinations of chemokines.

6.1. MATERIALS AND METHODS

6.1.1. ANIMALS AND REAGENTS

BALB/c mice are purchased from Harlan-Sprague-Dawley (Indianapolis, IN).

Human MDC (hMDC) was obtained from CD8⁺ T cell clones immortalized *in vitro* prepared as previously described (Markham et al., 1983, *Int. J. Cancer* 31:413; Markham et al. 1984, *Int. J. Cancer* 33:13). One such immortalized CD8⁺ T cell clone, F3b Clone 19, was adapted to growth in serum-free medium by the following procedure and used for further studies. F3b Clone 19 cells were grown in complete medium containing rIL-2 (16 ng/ml) at 37°C in a CO₂ incubator. After expanding the culture to 200 ml, the cells were pelleted and resuspended in RPMI medium containing HB101 (Irvine Scientific) supplemented with 16 ng/ml of rIL-2, 1% glutamine and 1% penicillin/streptomycin. The cells were grown to full confluence and the medium harvested by centrifugation at 670 x g for 10 minutes.

Human MDC (hMDC) was purified from F3b Clone 19 as described in Pal et al., 1997, *Science* 278:695-698. Briefly, the cell free culture supernatant from F3b Clone 19 was clarified by high speed centrifugation and fractionated by heparin affinity chromatography, taking advantage of the heparin binding characteristics of chemokines (Witt and Lander, 1994, *Current Biology* 4:394; Proost et al., 1996, *Method: A Companion to Methods in Enzymology* 10:82). Culture supernatant (1200 ml) from F3b Clone 19, grown to high cell density in serum-free medium supplemented with rIL-2 was clarified by high speed centrifugation (100,000 x g for 60 minutes at 4°C) and applied to a 5 ml HiTrap heparin affinity FPLC column (Pharmacia) equilibrated in 10 mM Tris-HCl, pH 7.6 containing 0.1 M NaCl (column buffer). The column was then washed extensively with column buffer and the bound proteins eluted from the column with 10 mM Tris-HCl, pH 7.6 containing 2.0 M NaCl at a flow rate of 0.5 to 1 ml/minute. Virtually all of the HIV suppressive activity effective against primary NSI and SI isolates and HIV-1_{MB} was recovered in the column eluate (data not shown). The heparin affinity column eluate was brought to pH 2.0 by addition of trifluoracetic acid (TFA) and subjected to reversed phase HPLC on a PEEK C-18 column (Waters Instruments) equilibrated in H₂O containing 0.1 % TFA. Proteins bound to the column were eluted with a 5 minute linear gradient of aqueous acetonitrile (0 to 35 %) containing 0.1% TFA. After 10 minutes at 35% acetonitrile, the column was further developed with a 60 minute linear gradient of 35-70% aqueous acetonitrile in TFA. The flow rate was maintained at 0.5 to 1 ml/minute. The fractions obtained were then tested for suppressor activity in the acute infectivity assay using HIV-1_{MB}. Active fractions were pooled, diluted twofold in H₂O with 0.1 % TFA.

and reapplied to the column. The column was then developed with a 30 minute linear aqueous acetonitrile gradient (0-60%) containing 0.1% TFA at a flow rate of 0.5 to 1 ml/minute. The fractions obtained were assayed as above. Active fractions were pooled, diluted with H₂O/0.1 % TFA and fractionated under the same conditions to obtain a single protein peak. The fraction corresponding to the peak and flanking fractions were tested in the infectivity assay to verify that suppressor activity was cofractionated with the protein.

Suppressive activity against HIV-1_{IIIB} in the absence of cytotoxic effects consistently copurified with a single protein peak that appeared as a homogeneous 8 kDa band when analyzed by SDS-polyacrylamide gel electrophoresis. This protein was not reactive in ELISAs for RANTES, MIP-1 α or MIP-1 β (R&D Systems).

Recombinant gp120 protein derived from HIV-1 IIIB isolate is purchased from Intracel (Foster City, CA).

6.1.2 IMMUNIZATION OF MICE

The hMDC and the gp120 is resuspended in a total volume of 50 μ l of phosphate-buffered saline (PBS). Mice are divided into 5 groups with 3-4 mice in each group. Groups 1-4 are inoculated with 10 μ g gp120 and 0.3 μ g, 0.1 μ g, 0.03 μ g, and 0.01 μ g of hMDC, respectively. As a control, group 5 is inoculated with 10 μ g of gp120 in the absence of hMDC. For primary inoculation, each group of mice is inoculated with 10 μ l of the hMDC and gp120 solution via footpad. Two to three weeks after the primary inoculation, each mouse is given the same doses of hMDC/gp120 that is used in primary inoculation.

6.1.3 ELISA ASSAY

Serum samples are collected one week after the second inoculation via tail vein bleed. gp120 serum responses are measured using standard gp120 antibody ELISA assays.

6.1.4 DTH ASSAY

The delayed-type hypersensitivity (DTH) response is measured from 1-7 days after the second inoculation. A caliper is to be used to measure footpad swelling.

6.2. RESULTS

Mice inoculated with hMDC/gp120 are expected to have greater serum antibody and DTH responses than mice inoculated with gp120 alone. The improved responses will be reflected in either increased titers of serum antibody responses or increased footpad swelling. A dose response effect is expected - increasing the dose of hMDC used is expected to cause a corresponding improvement in the serum and DHT gp120-specific responses.

7. EXAMPLE: OTHER CHEMOKINES AND COMBINATIONS OF CHEMOKINES

The foregoing experiments can be repeated using other chemokines and combinations of chemokines. For example, the experiments are preferably repeated using one or more chemokines selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

THE CLAIMS:

1. A method to enhance the efficacy of a vaccine in a subject comprising administering to the subject an immunogenic amount of one or more purified antigens against which an immune response is desired in the subject and an amount of one or more chemokines, or purified fragments or derivatives thereof, effective to enhance the efficacy of said vaccine.
2. The method of claim 1, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
3. The method of claim 1, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-

regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

4. The method of claim 1, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
5. The method of claim 1 wherein the fragment(s) or derivative(s) are truncation isoforms.
6. The method of claim 1, wherein the one or more chemokines include MDC comprising the amino acid sequence of SEQ ID NO: 2.
7. The method of claim 1, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2.
8. The method of claim 1, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2., which derivative has activity to enhance the efficacy of the vaccine.
9. The method of claim 1, wherein the one or more chemokine derivatives has one or more insertions or substitutions with one or more non-classical amino acids relative to a corresponding wildtype chemokine, which derivative has activity to enhance the efficacy of the vaccine.
10. The method of claim 1, including a chemokine derivative having one or more conservative substitutions in sequence relative a wildtype MDC, which derivative has activity to enhance the efficacy of the vaccine.
11. The method of claim 1, wherein the one or more chemokines include a human chemokine.

12. The method of claim 1, wherein the purified chemokine(s) or purified fragment(s) or derivative(s) thereof is/are administered concurrently with the purified antigen(s).
13. The method of claim 1 wherein the purified chemokine(s) or purified fragment(s) or derivative(s) thereof, are administered within a time period before or after administration of the purified antigen, which time period permits the purified MDC or purified fragment or derivative thereof MDC to enhance the efficacy of the vaccine.
14. The method of claim 1, wherein the antigen is an HIV antigen.
15. The method of claim 14, wherein the HIV antigen is HIV-associated gp120 protein.
16. The method of claim 1, wherein the subject is a human.
17. The method of claim 1, wherein the subject is infected or at risk of being infected with HIV virus.
18. The method of claim 1, wherein the vaccine elicits a humoral response against the antigen in the subject.
19. The method of claim 1, wherein the vaccine elicits a cell-mediated response against the antigen in the subject.
20. The method of claim 1, wherein the vaccine elicits both a humoral and a cell-mediated response against the antigen in the subject.
21. The method of claim 1, wherein the vaccine further comprises pharmaceutically acceptable excipient, auxiliary substance, adjuvant, wetting or emulsifying agent, or pH buffering agent.

22. A method to enhance the efficacy of a vaccine in a subject comprising administering to the subject a first amount of a first set of one or more purified nucleotide sequences encoding one or more antigens against which an immune response is desired in the subject and a second second set of one or more purified nucleic acids, each comprising a nucleotide sequence encoding one or more chemokines, or fragments or derivatives thereof, wherein the antigen(s) and the chemokine(s) are expressed in a coordinated manner upon introduction into a suitable cell, said first amount is immunogenic and said second amount is effective in enhancing the efficacy of the vaccine.
23. The method of claim 22, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
24. The method of claim 22, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine

1, HuMIC, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

25. The method of claim 22, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.

26. The method of claim 22 wherein the fragment(s) or derivative(s) are truncation isoforms.

27. The method of claim 22, wherein the nucleotide sequence encoding one or more chemokines comprises the nucleotide sequence of SEQ ID NO:1.

28. The method of claim 22, wherein one or more of the chemokine derivative(s) have deletional, insertional or substitutional mutations and combination thereof, which derivative has activity to enhance the efficacy of the vaccine.

29. The method of claim 22, wherein the vaccine elicits a humoral response against the antigen in the subject.

30. The method of claim 22, wherein the vaccine elicits a cell-mediated response against the antigen in the subject.

31. The method of claim 22, wherein the vaccine elicits both a humoral and a cell-mediated response against the antigen in the subject.

32. The method of claim 22, wherein the vaccine further comprises pharmaceutically acceptable excipient, auxiliary substance, adjuvant, wetting or emulsifying agent, or pH buffering agent.

33. A composition comprising: an immunogenic amount of one or more purified antigens and an amount of one or more purified chemokines, or purified

fragments or derivatives thereof, effective to enhance the immune response to said antigen(s); and a pharmaceutically acceptable carrier.

34. The composition of claim 33, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
35. The composition of claim 33, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
36. The composition of claim 33, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine, Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating

protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

37. The composition of claim 33, wherein the fragment(s) or derivative(s) are truncation isoforms.
38. The composition of claim 33, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2.
39. The composition of claim 33, wherein the one or more chemokine fragment includes an MDC fragment selected from the group consisting of amino acid numbers 2-69, 3-69, 5-69, 7-69 and 9-69 of SEQ ID NO: 2, which derivative has activity to enhance the efficacy of the vaccine.
40. The composition of claim 33, wherein the one or more chemokine derivatives has one or more insertions of or substitutions with one or more non-classical amino acids relative to a corresponding wildtype chemokine, which derivative has activity to enhance the efficacy of the vaccine.
41. The composition of claim 33, wherein the one or more chemokine derivatives has one or more conservative substitutions in sequence relative a corresponding wildtype chemokine, which derivative has activity to enhance the efficacy of the vaccine.
42. The composition of claim 33, wherein the chemokine is a human chemokine.
43. The composition of claim 33, wherein the antigen is an HIV antigen.
44. The composition of claim 43, wherein the antigen is HIV associated gp120 protein.
45. A composition comprising an amount of a first set of purified nucleic acids comprising one or more nucleotide sequences encoding one or more antigens

and a second set of purified nucleic acids comprising one or more nucleotide sequences encoding one or more chemokines, or fragments or derivatives thereof, wherein the antigen(s) and the chemokine(s), or fragment(s) or derivative(s) thereof, are expressed from said first set of nucleic acid(s) and second set of nucleic acid(s) in a coordinated manner such that upon introduction into a suitable cell, the amount of said first set of nucleic acid(s) is sufficient to express an immunogenic amount of the antigen and the amount of the said second set of nucleic acid(s) is effective in enhancing the efficacy of the vaccine; and a pharmaceutically acceptable carrier.

46. The composition of claim 45, wherein the chemokine is MDC and the nucleic acid encoding the MDC comprises the nucleotide sequence of SEQ ID NO: 1.
47. The composition of claim 45, wherein the chemokine derivative(s) have deletional, insertional or substitutional mutations and/or combinations thereof, and the derivative(s) have activity to enhance the efficacy of the vaccine.
48. The composition of claim 45, further comprising pharmaceutically acceptable excipient, auxiliary substance, adjuvant, wetting or emulsifying agent, or pH buffering agent.
49. A composition comprising a first set of purified nucleotide sequences encoding one or more antigens and a second set of purified nucleotide sequences encoding one or more chemokines, or fragments or derivatives thereof, wherein the antigen(s) and the chemokine(s) are expressed in a coordinated manner such that upon introduction into a suitable cell, the sets produce an amount of said antigen(s) that is immunogenic and an amount of chemokine(s), or fragment(s) or derivative(s) thereof, that is effective in enhancing the efficacy of the vaccine relative to a corresponding vaccine composition without such chemokine(s), fragment(s) or derivative(s) thereof.
50. The composition of claim 49, wherein the one or more chemokines are selected from the group consisting of: Macrophage-derived chemokine,

Monocyte chemotactic protein 1, Monocyte chemotactic protein 2, Monocyte chemotactic protein 3, Monocyte chemotactic protein 4, activated macrophage specific chemokine 1, Macrophage inflammatory protein 1 alpha, Macrophage inflammatory protein 1 beta, Macrophage inflammatory protein 1 gamma, Macrophage inflammatory protein 1 delta, Macrophage inflammatory protein 2 alpha, Macrophage inflammatory protein 3 alpha, Macrophage inflammatory protein 3 beta, Regulated upon activation, normal T cell expressed and secreted (and its variants), I-309, EBI1-ligand chemokine, Pulmonary and activation regulated chemokine, Liver and activation-regulated chemokine, Thymus and activation regulated chemokine, Eotaxin (and variants), Human CC chemokine 1, Human CC chemokine 2, Human CC chemokine 3, IL-10-inducible chemokine, liver-expressed chemokine, 6Ckine, Exodus 1, Exodus 2, Exodus 3, thymus-expressed chemokine, Secondary Lymphoid tissue chemokine, Lymphocyte and Monocyte chemoattractant; Monotactin, Activation-induced, chemokine-related molecule, Myeloid progenitor inhibitory factor-1, Myeloid progenitor inhibitory factor-2, Stromal cell-derived factor 1 alpha, Stromal cell-derived factor 1 beta, B-cell-attracting chemokine 1, HuMIG, H174, Interferon-stimulated T-cell alpha chemoattractant, Interleukin-8, IP-10, platelet factor 4, growth-regulated gene-alpha, growth-regulated gene-beta, growth-regulated gene-gamma, Neutrophil-activating protein 2, ENA-78, granulocyte chemotactic protein 2, LYMPHOTACTIN, and Fractalkine/neurotactin.

51. The method of claim 49, wherein the one or more chemokines are selected from a chemokine class selected from the group consisting of: CC, CXC, C-C and CX3C.
52. The method of claim 49, wherein the one or more chemokines are selected from the group consisting of: MDC, SDF-1, BLC, and MCP-1.
53. The composition of claim 49, wherein the fragment(s) or derivative(s) are truncation isoforms.

54. The composition of claim 49, wherein the nucleic acid is administered directly to the subject.
55. The composition of claim 49, wherein the nucleic acid is introduced into a suitable host cell and said suitable host cell is introduced into the subject.

GAGACATAACA	GGACAGAGCC	ATG	GCT	CGC	CTA	CAG	ACT	GCA	CTC	CTG	GTT	GTC	52			
Met	Ala	Arg	Leu	Gln	Thr	Ala	Leu	Leu	Val	Leu	Val	Val				
-24							-20					-15				
CTC	GTC	CTC	CTT	GCT	GTG	GCG	CTT	CAA	GCA	ACT	GAG	GCA	GGC	CCC	TAC	100
Leu	Val	Leu	Leu	Ala	Val	Ala	Leu	Gln	Ala	Thr	Glu	Ala	Gly	Pro	Tyr	
-10							-5								1	
GGC	GCC	AAC	ATG	GAA	GAC	AGC	GTC	TGC	TGC	CGT	GAT	TAC	GTC	CGT	TAC	148
Gly	Ala	Asn	Met	Glu	Asp	Ser	Val	Cys	Cys	Arg	Asp	Tyr	Val	Arg	Tyr	
5							10					15				
CGT	CTG	CCC	CTG	CGC	GTG	GTG	AAA	CAC	TTC	TAC	TGG	ACC	TCA	GAC	TCC	196
Arg	Leu	Pro	Leu	Arg	Val	Val	Lys	His	Phe	Tyr	Trp	Thr	Ser	Asp	Ser	
20							25					30				
TGC	CCG	AGG	CCT	GGC	GTG	GTG	TTG	CTA	ACC	TTC	AGG	GAT	AAG	GAG	ATC	244
Cys	Pro	Arg	Pro	Gly	Val	Val	Leu	Leu	Thr	Phe	Arg	Asp	Lys	Glu	Ile	
40							45								50	
TGT	GCC	GAT	CCC	AGA	GTG	CCC	TGG	GTG	AAG	ATG	ATT	CTC	AAT	AAG	CTG	292
Cys	Ala	Asp	Pro	Arg	Val	Pro	Trp	Val	Lys	Met	Ile	Leu	Asn	Lys	Leu	
55							60								65	

AGC CAA TGAAGAGCCT ACTCTGATGA CCGTGGCCCTT GGCTCCTCCA GGAGGCTCA
 Ser Gln

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GGAGCCCTAC CTCCCTGCCA TTATAGCTGC TCCCCGCCAG AAGCCTGTGC CAACTCTCTG 408
 CATTCCCTGA TCTCCATCCC TGTGGCTGTC ACCCTTGGTC ACCTCCGGTC TGTCACTGCC 468
 ATCTCCCCC TGACCCCTCT AACCCATCCT CTGCTCCCT CCCTGCAGTC AGAGGGTCCT 528
 GTTCCCATCA GCGATTCCCC TGCTTAAACC CTTCCCATGAC TCCCCACTGC CCTAAGGCTGA 588 6
 GGTCAAGTCTC CCAAGGCCTGG CATGTGGCCC TCTGGATCTG GGTTCCATCT CTGTCCTCCAG 648
 CCTGCCACT TCCCTTCATG AATGTTGGGT TCTAGCTCCC TGTTCCTCAA ACCCATACTA 708
 CACATCCAC TTCTGGGTCT TTGCCTGGGA TGTTGCTGAC ACTCAGAAAG TCCCACCCACC 768
 TGCACATGTG TAGCCCCACC AGCCCTCCAA GGCATTGCTC GCCCAAGCAG CTGGTAATTG 828
 CATTICATGT ATTAGATGTC CCCTGGCCCT CTGTCCTC TTAATAACCC TAGTCACAGT 888
 CTCCGGCAGAT TCTTGGGATT TGGGGGTTT CTCCCCCACC TCTCCACTAG TTGGACCAAG 948

FIG. 1A-2

<u>GT</u> <u>TTCTAGCT</u>	<u>AAGTTACTCT</u>	<u>AGTCTCCAAG</u>	<u>CCTCTAGCAT</u>	<u>AGAGCCTACTGC</u>	<u>AGACAGGGCCC</u>	<u>1008</u>
TGGCTCAGAA	TCAGGCCCCA	GAAAGTGGCT	GCAGACAAAA	TCAATAAAC	TAATGTCCT	1068
CCCCTCTCCC	TGCCAAAGG	CAGTTACATA	TCAATACAGA	GACTCAAGGT	CACTAGAAAT	1128
GGGCCAGCTG	GGTCAATGTG	AAGCCCCAA	TTTGCCAGA	TTCACCTTC	TTCCCCCACT	1188
CCCTTTTTT	TTTTTTTTT	TTTGAGATGG	AGTTTCGCTC	TTGTCACCCA	CGCTGGAGTC	1248
CAATGGTGTG	GTCTTGGCTT	ATTGAAGCCT	CTGCTCCTG	GGTTCAAGTG	ATTCTCTTGC	1308 3/6
CTCAGCCTCC	TGAGTAGCTG	GGATTACAGG	TTCCTGCTAC	CACGCCAAGC	TAATTTTGT	1368
ATTTTAGTA	GAGGACGAGGC	TTCACCATGT	TGGCCAGGCT	GGTCTCGAAC	TCCTGTCCCTC	1428
AGGTAATCCG	CCCACCTCAG	CCTCCAAAG	TGCTGGGATT	ACAGGGGTGA	GCCACAGTGC	1488
CTGGCCTCTT	CCCTCTCCCC	ACTGCCCTCC	CCAACTTTT	TTTTTTTTT	ATGGCAGGGT	1548
CTCACTCTGT	CGCCCAAGGCT	GGAGTGCAGT	GGGCTCACTA	CAACCTCGAC		1608
CTCCTGGGTT	CAAGTGATTTC	<u>TCCCACCCCCA</u>	<u>GCCTCCCCAAG</u>	<u>TAGCTGGGAT</u>	<u>TACAGGTGTG</u>	<u>1668</u>

FIG. IA-3

TGCCCACTACG GCTGGCTAAT TTTGTATT TAGTAGAGA CAGGTTTCAC CATATTGGCC 1728
AGGCTGGTCT TGAACTCCTG ACCTCAAGTG ATCCACCTTC CTGTGTGCTCC CAAAGTGCTG 1788
AGATTACAGG CGTGAGCTAT CACACCCAGC CTCCCCCTTT TTTCCCTAAT AGGAGACTCC 1848
TGTACCTTC TTCGTTTAC CTATGTCG TGTCTGCTTA CATTTCCTTC TCCCTCAGG 1908
CTTTTTGG GTGGTCCCTCC AACCTCCAAT ACCCAGGCCT GGCCCTCTCA GAGTACCCCC 1968
CATTCCACT TCCCTGCCTC CTTCCCTAAA TAGCTGACAA TCAAATTCAAT GCTATGGTGT 2028 4/6
GAAAGACTAC CTITGACTTG GTATTATAAG CTGGAGTTAT ATATGTATT GAAACAGAG 2088
TAAATACTTA AGAGGCCAAA TAGATGAATG GAAGAATTAGGAACTGTG AGAGGGGGAC 2148
AAGGTGAAGC TTTCCTGCC CTGGGAGGAA GCTGGCTGTG GTAGCGTAGC GCTCTCTCTC 2208
TCTGTCTGTG GCAGGAGCCA AAGAGTAGGG TGTAAATTGAG TGAAAGGAATC CTGGGTAGAG 2268
ACCATTCTCA GGTGGTTGGG CCAGGGCTAAA GACTGGGAGT TGGGTCTATC TATGCCTTTC 2328
TGGCTGATT TTGTAGAGAC GGGGTTTGTGC CATGTTACCC AGGCTGGTCT CAAACTCCCTG 2388

FIG. 1A-4

GGCTCAAGCG ATCCCTGGG CTCAGCCTCC CAAAGTGTG GGATTACAGG CGTGAAATCAC 2448
 TGCGGCTGGC TTCCCTTCC TCTTGAGAAA TATTCTTTTC ATACAGCAAG TATGGGACAG 2508
 CAGTGTCCCA GGTAAAGGAC ATAATGTAA CAAGTGTCTG GTCCCTTCTG AGGGAGGCTG 2568
 GTGCCGCTCT GCAGGGTATT TGAACCTGTA GAATTGGAGG AGGCCATTTC ACTCCCTGAA 2628
 CCCAGCCTGA CAAATCACAG TGAGAAATGTT CACCTTATAG GCTTGCTGTG GGGCTCAGGT 2688
 TGAAAGTGTG GGGAGTGACA CTGCCTAGGC ATCCAGCTCA GTGTCATCCA GGGCCTGTGT 2748^{5/6}
 CCCTCCCGAA CCCAGGGTCA ACCTGCCTGC CACAGGCACT AGAAGGACGA ATCTGCCTAC 2808
 TGCCCATGAA CGGGGCCCTC AAGCGTCCCTG GGATCTCCTT CTCCTCTG TCCTGTCCCT 2868
 GCCCCCTCAGG ACTGCTGGAA ATAATATCCT TTAAAATAGT AAAAAGAAA AAAA 2923

FIG. IA-5

FIG. IA-1
FIG. IA-2
FIG. IA-3
FIG. IA-4
FIG. IA-5

FIG. IA

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Met	Ala	Arg	Leu	Gln	Thr	Ala	Leu	Val	Val	Leu	Val	Leu	Leu	Ala	
-24		-20												-10	
Val	Ala	Leu	Gln	Ala	Thr	Glu	Ala	Gly	Pro	Tyr	Gly	Ala	Asn	Met	Glu
		-5													
Asp	Ser	Val	Cys	Cys	Arg	Asp	Tyr	Val	Arg	Tyr	Arg	Leu	Pro	Leu	Arg
		10				15									
Val	Lys	His	Phe	Tyr	Tyr	Trp	Thr	Ser	Asp	Ser	Cys	Pro	Arg	Pro	Gly
25				30					35						40
Val	Val	Leu	Leu	Thr	Phe	Arg	Asp	Lys	Glu	Ile	Cys	Ala	Asp	Pro	Arg
									50						55
Val	Pro	Trp	Val	Lys	Met	Ile	Leu	Asn	Lys	Leu	Ser	Gln			
									65						

FIG. 1B

GGAGCCCTAC	CTCCCTGCCA	TTATAGCTGC	TCCCCGCCAG	AAGCTGTGC	CAACTCTCTG	408
CATTCCCTGA	TCTCCATCCC	TGTGGCTGTC	ACCCCTGGTC	ACCTCCGTGC	TGTCACTGCC	468
ATCTCCCCC	TGACCCCTCT	AACCCATCCT	CTGCCTCCCT	CCCTGCAGTC	AGAGGGTCCT	528
GTTCCCATCA	GCGATTCCCC	TGCTTAAACC	CTTCCATGAC	TCCCCACTGC	CCTAAGCTGA	588
GGTCAGTCTC	CCAAGCCTGG	CATGTGGCCC	TCTGGATCTG	GGTTCCATCT	CTGTCTCCAG	648
CCTGCCCACT	TCCCCTCATG	AATGTGGGT	TCTAGCTCCC	TGTTCTCCAA	ACCCATACTA	708
CACATCCAC	TTCTGGGTCT	TTGCCCTGGGA	TGTTGCTGAC	ACTCAGAAAG	TCCCACCAAC	768
TGCACATGTC	TAGCCCCACC	GGACCCCTCAA	GGCATTGCTC	CCCCAAGCAG	CTGGTAATTTC	828
CATTTCATGT	ATTAGATGTC	CCCTGGCCCT	CTGTCCCTC	TTAATAACCC	TAGTCACAGT	888
CTCCGCAGAT	TCTTGGGATT	TGGGGGTTT	CTCCCCCACC	TCTCCACTAG	TTGGACCAAG	948
GTTTCTAGCT	AAGTTACTCT	AGTCTCCAAG	CCTCTAGCAT	AGAGCACTGC	AGACAGGCC	1008
TGGCTCAGAA	TCAGAGCCA	GAAGTGGCT	GCAGACAAA	TCATAAAAC	TAATGTCCCT	1068
CCCCCTCTCC	TGCCAAAAGG	CAGTTACAT	TCAATACAGA	GAATCAAGGT	CACTAGAAAT	1128
GGGGCAGCTG	GGTCAATGTC	AAGCCCCAAA	TTTGCCCAAGA	TTCACCTTTC	TTCCCCCACT	1188
CCCCTTTTTG	TTTTTTTTTT	TTTGAGATGG	AGTTTCGCTC	TTGTCACCCA	CGCTGGAGTG	1248
CAATGGTGTG	GTCTGGCTT	ATTGAAGCT	CTGCCCTCTG	GGTTCAAGTG	ATTCTCTTGC	1308
CTCAGCCTCC	TGAGTAGCTG	GGATTACAGG	TTCTGCTAC	CACGCCAGC	TAATTTTTGT	1368
ATTTTTAGTA	GAGACGAGGC	TTCACCATGT	TGCCCAAGGCT	GGTCTGAAC	TCCTGTCCCTC	1428
AGGTAATCCG	CCCCACCTCG	CCTCCCAAAG	TGCTGGGATT	ACAGGCCTGA	GCCACAGTGC	1488
CTGGCCTCTT	CCCTCTCCCC	ACTGCCCTCC	CCAACTTTT	TTTTTTTTT	ATGGCAGGGT	1548
CTCACTCTG	CGCCCCAGGT	GGAGTGCAGT	GGCGTGTACT	CGGCTCACTA	CAACCTCGAC	1608
CTCCTGGTT	CAAGTGATTC	TCCCACCCCA	GCCTCCCAAG	TAGCTGGGAT	TACAGGTGTG	1668
TGCCACTACG	GCTGGCTAA	TTTGTATTT	TTAGTAGAGA	CAGGTTTCAC	CATATTGGCC	1728
AGGCTGGTCT	TGAACCTCTG	ACCTCAAGTG	ATCCACCTTC	CTTGTGCTCC	CAAAGTGCTG	1788
AGATTACAGG	CGTGAACCTAT	CACACCCAGC	CTCCCCCTTT	TTTTCTTAAT	AGGAGACTCC	1848
TGTACCTTTC	TTCTGTTTAC	CTATGTGTCG	TGTCCTGCTTA	CATTTCTTC	CCCCCTCAGG	1908
CTTTTTTGTG	GTGGCTCTTC	AACCTTCAAT	ACCCAGGCT	GGCCTCTTCA	GAGTACCCCC	1968
CATTCACCTT	TCCCTGCCCTC	CTTCCTTAAA	TAGCTGACAA	TCAAATTCTAT	GCTATGGTGT	2028
GAAAGACTAC	CTTTGACTTG	GTATTATAAG	CTGGAGTTAT	ATATGTATTT	AAAACAGAG	2088
TAAGTACTTA	AGAGGCCAAA	TAGATGAATG	GAAGAATTTT	AGGAACCTGTG	AGAGGGGGAC	2148
AAGGTGAAGC	TTTCTGGCC	CTGGGAGGAA	GCTGGCTGTG	GTAGCGTAGC	GCTCTCTCTC	2208
TCTGCTGTG	GCAGGAGGCC	AAGACTAGGG	TGTAATTGAG	TGAAGGAATC	CTGGGTAGAG	2268
ACCATTCTCA	GGTGGTTGGG	CCAGGCTAAA	GACTGGGAGT	TGGGTCTATC	TATGCCCTTTC	2328
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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 93 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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C-CHEMOKINES
LYMPHOTACTIN

(SCM-1)

D63789 D63790

CX3C-chemokines
Fractalkine/neurotactin

U91835 U84487

LOCUS HSU83171 2923 bp mRNA PRI 31-MAY-1997
 DEFINITION Human macrophage-derived chemokine precursor (MDC) mRNA,
 complete
 cds.
 ACCESSION U83171
 NID g1931580
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (bases 1 to 2923)
 AUTHORS Godiska,R., Chantry,D., Raport,C.J., Sozzani,S., Allavena,P.,
 Leviten,D., Mantovani,A. and Gray,P.W.
 TITLE Human macrophage-derived chemokine (MDC), a novel
 chemoattractant for monocytes, monocyte-derived dendritic cells, and natural
 killer cells
 JOURNAL J. Exp. Med. 185 (9), 1595-1604 (1997)
 MEDLINE 97296313
 REFERENCE 2 (bases 1 to 2923)
 AUTHORS Godiska,R. and Gray,P.W.
 TITLE Direct Submission
 JOURNAL Submitted (23-DEC-1996) ICOS Corporation, 22021 20th Avenue SE,
 Bothell, WA 98021, USA
 FEATURES Location/Qualifiers
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 /db_xref="taxon:9606"
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// LOCUS HSU83239 932 bp mRNA PRI 02-MAY-1997
DEFINITION Human CC chemokine STCP-1 mRNA, complete cds.
ACCESSION U83239
NID g2062424
KEYWORDS .
SOURCE human.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 932)
AUTHORS Chang,M.S., McNinch,J., Elias III,C., Manthey,C.L.,
Grosshans,D.,
TITLE Meng,T., Boone,T. and Andrew,D.P.
Molecular cloning and functional characterization of a novel CC
chemokine STCP-1 which specifically acts on activated T
lymphocytes
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 932)
AUTHORS Chang,M.S., McNinch,J., Elias III,C., Manthey,C.L.,
Grosshans,D.,
TITLE Meng,T., Boone,T. and Andrew,D.P.
JOURNAL Direct Submission
Submitted (26-DEC-1996) Research Computing, Amgen Institute,
620 University Ave, Suite 706, Toronto, ON M5G 2C1, Canada
FEATURES Location/Qualifiers
source 1..932
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LOCUS HSMCP1 725 bp RNA PRI 03-APR-1995
 DEFINITION H.sapiens mRNA for monocyte chemoattractant protein 1 (MCP-1).
 ACCESSION X14768
 NID g34513
 KEYWORDS monocyte chemoattractant protein 1.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (bases 1 to 725)
 REFERENCE Yoshimura,T., Yuhki,N., Moore,S.K., Appella,E., Lerman,M.I. and
 AUTHORS Leonard,E.J.
 TITLE Human monocyte chemoattractant protein-1 (MCP-1). Full-length
 cDNA
 JOURNAL cloning, expression in mitogen-stimulated blood mononuclear
 leukocytes, and sequence similarity to mouse competence gene JE
 FEBS Lett. 244 (2), 487-493 (1989)
 MEDLINE 89153605
 COMMENT ZAPII.
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  181 ttcctgtca gaggctcgac agctatagaa gaatcaccag cagcaagtgt cccaaagaag
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LOCUS HSMCP2 2991 bp DNA **PRI** 20-MAR-1997
DEFINITION *H.sapiens* MCP-2 gene.
ACCESSION X99886
NID g1905800
KEYWORDS MCP-2 gene; monocyte chemotactic protein 2; SCYA10 gene.
SOURCE human.
ORGANISM *Homo sapiens*
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 2991)
AUTHORS Van Coillie,E., Fiten,P., Nomiyama,H., Sakaki,Y., Miura,R.,
 Yoshie,O., Van Damme,J. and Opdenakker,G.
TITLE The human MCP-2 gene (SCYA8): cloning, sequence analysis,
 tissue expression, and assignment to the CC chemokine gene contig on
 chromosome 17q11.2
JOURNAL Genomics 40 (2), 323-331 (1997)
MEDLINE 97237052
REFERENCE 2 (bases 1 to 2991)
AUTHORS Opdenakker,G.M.M.
TITLE Direct Submission
JOURNAL Submitted (07-AUG-1996) G.M.M. Opdenakker, Rega Institute for
 Medical Research, Minderbroedersstraat 10, B 3000 Leuven,
BELGIUM
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//LOCUS HSY16645 1368 bp mRNA PRI 25-SEP-1998

DEFINITION Homo sapiens mRNA for monocyte chemotactic protein-2.

ACCESSION Y16645

NID g2916795

KEYWORDS MCP-2 gene; monocyte chemotactic protein 2.

SOURCE human.

ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1368)

AUTHORS Van Coillie,E.

TITLE Functional comparison of two human monocyte chemotactic protein-2 isoforms, role of the amino-terminal pyroglutamic acid and processing by CD26/dipeptidyl peptidase IV

JOURNAL Biochemistry 37, 12672-12680 (1998)

REFERENCE 2 (bases 1 to 1368)

AUTHORS Van Coillie,E.

TITLE Direct Submission

JOURNAL Submitted (23-FEB-1998) E. Van Coillie, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, BELGIUM

COMMENT Related sequences: X99886, Y10802.

FEATURES Location/Qualifiers

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sig_peptide 473..541
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CDS 473..772
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variation 677
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BASE COUNT 457 a 292 c 243 g 376 t

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 1201 ctgtatataaa ctatggatt ttgaaaaaaaaa atttcaaaaa gaaaaaaaaata tatataattt
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 1321 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 //LOCUS HSMCP3A 1085 bp DNA PRI 25-JUL-1994
 DEFINITION H.sapiens MCP-3 mRNA for monocyte chemotactic protein-3.
 ACCESSION X72308 S57464
 NID g313707
 KEYWORDS monocyte chemotactic protein 3.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (bases 1 to 1085)
 REFERENCE Opdenakker,G., Froyen,G., Fiten,P., Proost,P. and Van Damme,J.
 AUTHORS
 TITLE Human monocyte chemotactic protein-3 (MCP-3): molecular cloning
 of
 JOURNAL the cDNA and comparison with other chemokines
 MEDLINE Biochem. Biophys. Res. Commun. 191 (2), 535-542 (1993)
 93213290
 REFERENCE 2 (bases 1 to 1085)
 AUTHORS Opdenakker,G.M.
 TITLE Direct Submission
 JOURNAL Submitted (27-MAY-1993) G.M. Opdenakker, Rega Institute,
 University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, BELGIUM
 3 (bases 1 to 1085)
 REFERENCE Opdenakker,G., Fiten,P., Nys,G., Froyen,G., Van Roy,N.,
 AUTHORS Speleman,F., Laureys,G. and Van Damme,J.
 TITLE The human MCP-3 gene (SCYA7): cloning, sequence analysis, and
 assignment to the C-C chemokine gene cluster on chromosome
 17q11.2-q12
 JOURNAL Genomics 21 (2), 403-408 (1994)
 MEDLINE 94375065
 FEATURES Location/Qualifiers
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 /organism="Homo sapiens"
 /db_xref="taxon:9606"
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 CDS 299..628
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 /db_xref="SWISS-PROT:P80098"
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 BASE COUNT 314 a 214 c 229 g 328 t

ORIGIN

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181 gtccttttg atcgtggtaa tttagagggt gaactcaactg gaatggggat gcttgcgtgt
241 gtaatcttac taagagctaa tagaaaggctt aggaccaaac cagaaacactc caattctcat
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421 aactacactgc tgctacagat ttatcaataa gaaaatccct aagcagagggc tggagagacta
481 cagaaggacc accagtagcc actgtttcccg ggaagctgtt atcttcaaga ccaaactggaa
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601 caagaaaacc caaatccaa agcttgaac attcatactg gaactgaaaaa caagccatgtt
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961 aattgttaag atatgtatgtc cctatggaa catattgtt ttatataattt acatatttgc
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LOCUS      HSMCP3A      1085 bp      DNA      PRI      25-JUL-1994
DEFINITION H.sapiens MCP-3 mRNA for monocyte chemotactic protein-3.
ACCESSION  X72308 S57464
NID        g313707
KEYWORDS   monocyte chemotactic protein 3.
SOURCE     human.
ORGANISM   Homo sapiens
Eukaryotes; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 1085)
AUTHORS    Opdenakker,G., Froyen,G., Fiten,P., Proost,P. and Van Damme,J.
TITLE      Human monocyte chemotactic protein-3 (MCP-3): molecular cloning
of
JOURNAL   the cDNA and comparison with other chemokines
MEDLINE   Biochem. Biophys. Res. Commun. 191 (2), 535-542 (1993)
93213290
REFERENCE  2 (bases 1 to 1085)
AUTHORS    Opdenakker,G.M.
TITLE      Direct Submission
JOURNAL   Submitted (27-MAY-1993) G.M. Opdenakker, Rega Institute,
University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, BELGIUM
REFERENCE  3 (bases 1 to 1085)
AUTHORS    Opdenakker,G., Fiten,P., Nys,G., Froyen,G., Van Roy,N.,
Speleman,F., Laureys,G. and Van Damme,J.
TITLE      The human MCP-3 gene (SCYA7): cloning, sequence analysis, and
assignment to the C-C chemokine gene cluster on chromosome
17q11.2-q12
JOURNAL   Genomics 21 (2), 403-408 (1994)
MEDLINE   94375065
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source    1..1085
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gene      299..810
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CDS       299..628
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KTQTPKL"
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sig_peptide 299..397
           /gene="MCP-3"
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mat_peptide 398..625
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polyA_signal 806..810
 BASE COUNT 314 a 214 c 229 g 328 t
 ORIGIN
 1 ggtttctatt gacttgggtt aatcgtgtga cgcgggtggc tggcacgaaa ttgaccaacc
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 241 gtaatcttac taagagctaa tagaaaggct aggaccaaac cagaaaccc tcattctcat
 301 gtggaaagccc atgccttcac cctccaaat gaaaggctct gcagcaccc tgcgtctgt
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 481 cagaaggacc accagtagcc actgtccccg ggaagctgta atcttcaga ccaaaactgg
 541 caaggagatc tgcgtgtacc ccacacagaa gtgggtccag gactttatgaa agcacctgg
 601 caagaaaacc caaactccaa agcttgaac attcatgact gaactgaaaaa caagccatgaa
 661 cttgagaaac aaataatttt tataccctgt ctttctca gtcgttctg agattatttt
 721 aatctaattc taaggatataatgat tataatgtt aatcatgtt tttcttagta
 781 gatattaaaaa gtttataatataat ttttataatataatcatttcatg gatattttgtt ggtttgtt
 841 ataaagccctt ggatgtatataat gtcatctcag tgcgttataaa actgtggat gtcctccct
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 961 aattgttaag atatgtatgtc cctatggaaat catattgttataat tataatataat acatatttgc
 1021 atatgtatgaa cttccaaattt ttcacataaa atagatttt gtataacaaa aaaaaaaaaaaa
 1081 aaaaaa
 //LOCUS HSU46767 825 bp mRNA PRI 16-DEC-1996
 DEFINITION Human monocyte chemoattractant protein-4 precursor (MCP-4)
 mRNA,
 ACCESSION complete cds.
 NID U46767
 g1732122
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 825)
 AUTHORS Garcia-Zepeda, E.A., Combadiere, C.C., Rothenberg, M.E.,
 Sarafi, M.N.,
 TITLE Lavigne, F., Hamid, Q., Murphy, P. and Luster, A.D.
 Human monocyte chemoattractant Protein (MCP)-4: A novel CC
 chemokine with activities on monocytes, eosinophils, and
 basophils
 induced in allergic and non-allergic inflammation that signals
 through the CC chemokine receptors CCR-2 and 3
 J. Immunol. 158 (1996) In press
 2 (bases 1 to 825)
 REFERENCE Garcia-Zepeda, E.A. and Luster, A.D.
 AUTHORS Direct Submission
 TITLE Submitted (22-JAN-1996) Eduardo A. Garcia-Zepeda, Infectious
 JOURNAL Disease Unit, Massachusetts General Hospital, 149 13th St.,
 Charlestown, MA 02129, USA
 FEATURES Location/Qualifiers
 source 1..825
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /tissue_type="heart"
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 CDS 34..330
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 subfamily signature; chemoattractant for monocytes, eosinophils"
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 /product="monocyte chemoattractant protein-4"
 precursor /db_xref="PID:g1732123"
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LQRLKSYVITTSRCPQKAVIFRTKLGKEICADPKEKWWQNYMKHLGRKAHTLKT"

gene 34..330
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mat_peptide 103..327
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BASE COUNT 221 a 175 c 185 g 244 t

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 181 agctatgtga tcaccacccag caggtgtccc cagaaggctg tcatcttcg aaccaaactg
 241 ggcaaggaga tctgtgtga cccaaaggag aagtgggtcc agaattatata gaaacacctg
 301 ggccggaaag ctcacacccctc gaagacttga actctgctac ccctactgaa atcaagctgg
 361 agtacgtgaa atgacttttc cattctccctc tggcctccctc ttctatgctt tggaaatactt
 421 ctaccataat tttcaaatag gatgcattcg gtttgtgtat tcaaaatgtt cttatgttta
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 661 agtcatggac atgaagggtt gctgcaatgtt aggaaggaga gctcttgcg aatgtgagg
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//LOCUS HSAMAC1 803 bp RNA PRI 10-AUG-1997

DEFINITION Homo sapiens mRNA for alternative activated macrophage specific CC

ACCESSION Y13710

NID g2326515

KEYWORDS AMAC-1 gene; CC-chemokine 1.

SOURCE human. ORGANISM Homo sapiens
 Eukaryotes; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.

REFERENCE 1 (bases 1 to 803)

AUTHORS Politz,O.

TITLE Direct Submission

JOURNAL Submitted (10-JUN-1997) Politz O., Dermatology, Free University
 Benjamin Franklin Medical Center, Hindenburgdamm 30; 12200
 Berlin

REFERENCE 2 (bases 1 to 803)

AUTHORS Kodelja,V., Mueller,C., Politz,O., Hakiy,N., Orfanos,C.E. and
 Goerdt,S.

TITLE Cloning of alternative activated macrophage associated CC
 chemokine

JOURNAL 1 (AMAC-1)

FEATURES Unpublished

source Location/Qualifiers

1..803
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
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sig_peptide 71..133
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CDS 71..340
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 /db_xref="PID:e321838"
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/translation="MKGLAAALLVLVCTMALCSAQVGTNKELCCLVYTSWQIPQKFI
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gene 71..340
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BASE COUNT 214 a 213 c 160 g 216 t

ORIGIN

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661 cagacattgt gccatatgta tcaaatacga aatctttt gatggttt gtcagcacc
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781 aaaaaaaaaa aaaaaaaaaa aaa

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LOCUS HUMLD78A 3176 bp DNA **PRI** 17-JAN-1992
DEFINITION Human LD78 alpha gene.
ACCESSION D90144
NID g219905
KEYWORDS LD78; LD78 alpha; cytokine; inducible gene family; secreted peptide.
SOURCE Human blood lymphocyte DNA, clone Lm LD-3.
ORGANISM Homo sapiens
Eukaryota; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 1 to 3176)
AUTHORS Nakao, M., Nomiyama, H. and Shimada, K.
TITLE Structures of human genes coding for cytokine LD78 and their expression
JOURNAL Mol. Cell. Biol. 10 (7), 3646-3658 (1990)
MEDLINE 90287155
COMMENT These data kindly submitted in computer readable form by:
Hisayuki Nomiyama
Department of Biochemistry
Kumamoto University Medical School
2-2-1 Honjo, Kumamoto 860
Japan
Phone: 096-344-2111
Fax: 096-372-6140.
FEATURES Location/Qualifiers
source 1..3176
/organism="Homo sapiens"
/db_xref="taxon:9606"
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exon 1069..1227
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sig_peptide 1155..1220
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CDS join(1155..1227,1916..2030,2451..2541)
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/db_xref="PID:g219906"

/translation="MQVSTAALAVLLCTMALCNQFSASLAADTPTACCFSYTSRQIPQ
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intron 1228..1915
exon 1916..2030
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intron 2031..2450
exon 2451..2957
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BASE COUNT 833 a 741 c 752 g 850 t
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  181 caattcttca gactttgtag aatttgcata atgtcgatc ttgtttttt taaccactga
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  301 tggataattc atcattccct gatggagaaa aatttagctt tggttattttt agagttataaa
  361 acgatgtgg gtcaggatc ttatgtttt aagatggctt catatttggg ttgtttccac
  421 agaactctt cctagaatg cttttcttag gttatggctt acagatattt cttaggcacct
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  2821 aaccgagtttctgatc cctgtgttagg cagttcatggc accaaaccca ccagactgac
  2881 aatgtgtatc cggatgttttgc tgggttgc tgggttgc tgggttgc aataaagatg
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  3001 ctgggtttaaaa ggaatcatag gcaaaagatgg ggaagaggtt aatggaggg aaattgggag
  3061 agatggggag ggcttaccaca gagttatcca ctttacaacg gagacacagt tctggacat
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LOCUS AF043339 225 bp mRNA PRI 23-FEB-1998
 DEFINITION Homo sapiens macrophage inflammatory protein 1 alpha (MIP1 α)
 mRNA,
 partial cds.
 ACCESSION AF043339
 NID g2905627
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 225)
 AUTHORS Jang, J.S. and Kim, B.E.
 TITLE Direct Submission
 JOURNAL Submitted (15-JAN-1998) Protein Engineering, General Institute

of

Technology, Hyundai Pharm. Ind. Co., Ltd., 213 Sosa Bon 1-dong, Sosa-gu, Bucheon 422-231, Korea

COMMENT forward primer (5'-tgcgcatacttgctgctgaca-3')
reverse primer (5'-cttctggaccctcaggcact-3').

FEATURES Location/Qualifiers

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cycles; DeltaCycler II from Ericomp
CDS <1..213
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/function="CC chemokine"
/function="proinflammatory cytokine involved in inflammation"
/note="8-10 kDa"
/codon_start=1
/product="macrophage inflammatory protein 1 alpha"
/db_xref="PID:g2905628"

/translation="ASLAADTPTACCFSYTSRQIPQNFIAODYFETSSQCSKPGVIFLT
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primer_bind complement(205..225)
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BASE COUNT 50 a 68 c 62 g 45 t

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181 aaatatgtca gcgaccttgg a gtcgtgtcc tgagggttcc agaag

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LOCUS HUMLD78B 3112 bp DNA PRI 17-JAN-1992

DEFINITION Human LD78 beta gene.

ACCESSION D90145

NID 9219907

KEYWORDS LD78; LD78 beta; cytokine; inducible gene family; secreted peptide.

SOURCE Human placenta DNA, clone Lm LD-1.

ORGANISM Homo sapiens

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;

Hominidae; Homo.

REFERENCE 1 (bases 1 to 3112)

AUTHORS Nakao, M., Nomiyama, H. and Shimada, K.

TITLE Structures of human genes coding for cytokine LD78 and their expression

JOURNAL Mol. Cell. Biol. 10 (7), 3646-3658 (1990)

MEDLINE 90287155

COMMENT These data kindly submitted in computer readable form by:
Hisayuki Nomiyama
Department of Biochemistry
Kumamoto University Medical School
2-2-1 Honjo, Kumamoto 860
Japan
Phone: 096-344-2111
Fax: 096-372-6140.

FEATURES Location/Qualifiers

source 1..3112
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/db_xref="taxon:9606"
repeat_unit 498..797
/note="Alu repeat"

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TATA_signal      1078..1082
prim_transcript 1106..2995
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exon            1106..1267
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CDS             join(1192..1267,1953..2067,2488..2578)
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/db_xref="PID:g219908"


sig_peptide      1192..1260
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mat_peptide      join(1258..1267,1953..2067,2488..2575)
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intron          1268..1952
exon            1953..2067
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intron          2068..2487
exon            2488..2955
/number=3

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 LOCUS HUMACT2A 696 bp mRNA PRI 30-OCT-1994
 DEFINITION Human activation (Act-2) mRNA, complete cds.
 ACCESSION J04130
 NID g178017
 KEYWORDS act2 gene; immune activation gene.
 SOURCE Human (Hut-102B2 library) activated T cells, cDNA to mRNA.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 696)
 AUTHORS Lipes,M.A., Napolitano,M., Jeang,K.T., Chang,N.T. and
 Leonard,W.J.
 TITLE Identification, cloning, and characterization of an immune
 activation gene
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 85 (24), 9704-9708 (1988)
 MEDLINE 89071764
 COMMENT Draft entry and computer-readable sequence [1] kindly submitted
 by W.Leonard, 09-JAN-1989.
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 /db_xref="taxon:9606"
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 541 gtcatttcca ttatattat tagtttagcc aaaggataag tgtcctatgg ggatggtcca
 601 ctgtcactgt ttctctgttgc ttgcaataac atggataaca catttggattc tgtgtgtttt
 661 ccataataaa actttaaaat aaaatgcaga cagtt
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 LOCUS AF031587 481 bp mRNA PRI 02-JAN-1998
 DEFINITION Homo sapiens MIP-1 delta mRNA, complete cds.

ACCESSION AF031587
 NID g2739163
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 481)
 AUTHORS Wang,W.
 TITLE Molecular cloning and characterization of a new CC chemokine
 MIP-1
 JOURNAL delta
 Unpublished
 REFERENCE 2 (bases 1 to 481)
 AUTHORS Wang,W.
 TITLE Direct Submission
 JOURNAL Submitted (27-OCT-1997) Immunobiology, DNAX Research Institute,
 901 California Ave, Palo Alto, CA 94304, USA
 FEATURES Location/Qualifiers
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 /db_xref="taxon:9606"
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 DCMKKLKPYSI"
 BASE COUNT 140 a 112 c 100 g 129 t
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 361 gggcagccac ccacccctcaa cacccctgt gagtttcttg gtctgaaata cttaaaaaaat
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 LOCUS AF043340 234 bp mRNA PRI 23-FEB-1998
 DEFINITION Homo sapiens macrophage inflammatory protein 2 alpha (MIP2a)
 mRNA.
 partial cds.
 ACCESSION AF043340
 NID g2905629
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 234)
 AUTHORS Jang,J.S. and Kim,B.E.
 TITLE Direct Submission
 JOURNAL Submitted (15-JAN-1998) Protein Engineering, General Institute
 of
 Technology, Hyundai Pharm. Ind. Co., Ltd., 213 Sosa Bon 1-dong,
 Sosa-gu, Bucheon 422-231, Korea
 COMMENT forward primer (5'-tgcgcacccctggccactgaactg-3')
 reverse primer (5'-ccttccttctggcagtgg-3').
 FEATURES Location/Qualifiers
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      /function="proinflammatory cytokine involved in
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      /note="8-10 kDa"
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      /db_xref="PID:g2905630"



LOCUS HSU77035 764 bp mRNA PRI 23-JAN-1997



DEFINITION Human macrophage inflammatory protein 3 alpha (MIP-3a) mRNA, complete cds.



ACCESSION U77035



NID g1790924



KEYWORDS



SOURCE human.



ORGANISM Homo sapiens



Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.



REFERENCE 1 (bases 1 to 764)



AUTHORS Rossi,D.L., Vicari,A.P., Franz-Bacon,K., McClanahan,T.K. and Zlotnik,A.



TITLE Identification through bioinformatics of two new macrophage proinflammatory human chemokines: MIP-3alpha and MIP-3beta



JOURNAL J. Immunol. 158 (3), 1033-1036 (1997)



MEDLINE 97166046



REFERENCE 2 (bases 1 to 764)



AUTHORS Rossi,D.L. and Zlotnik,A.



TITLE Direct Submission



JOURNAL Submitted (31-OCT-1996) Immunology, DNAX Research Institute, 901



FEATURES California Ave., Palo Alto, CA 94304, USA



source Location/Qualifiers



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/organism="Homo sapiens"



/db_xref="taxon:9606"



/cell_type="elutriated monocytes activated with LPS/IFN-GAMMA"



gene 1..291



/gene="MIP-3a"



CDS 1..291



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/note="chemokine"



/codon_start=1



/product="macrophage inflammatory protein 3 alpha"



/db_xref="PID:g1790925"



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721 aaatattaat aagacaaaata ttgaaaataa agaaaacaaaa agtt

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LOCUS HSU77180 545 bp mRNA PRI 23-JAN-1997  

DEFINITION Human macrophage inflammatory protein 3 beta (MIP-3beta) mRNA,  

complete cds.  

ACCESSION U77180  

NID g1791002  

KEYWORDS  

SOURCE human.  

ORGANISM Homo sapiens  

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  

Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  

REFERENCE 1 (bases 1 to 545)  

AUTHORS Rossi,D.L., Vicari,A.P., Franz-Bacon,K., McClanahan,T.K. and  

Zlotnik,A.  

TITLE Identification through bioinformatics of two new macrophage  

proinflammatory human chemokines: MIP-3alpha and MIP-3beta  

JOURNAL J. Immunol. 158 (3), 1033-1036 (1997)  

MEDLINE 97166046  

REFERENCE 2 (bases 1 to 545)  

AUTHORS Vicari,A. and Zlotnik,A.  

TITLE Direct Submission  

JOURNAL Submitted (01-NOV-1996) Immunology, DNAX Research Institute,  

901 California Ave, Palo Alto, CA 94304, USA  

FEATURES Location/Qualifiers  

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CDS 1..297  

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181 gtgttccacca cactgagggg cggccagctc tttgcacccc cagaccagcc ctgggttagaa  

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LOCUS HUMTCSM 1160 bp mRNA PRI 15-JUN-1989  

DEFINITION Human T cell-specific protein (RANTES) mRNA, complete cds.  

ACCESSION M21121  

NID g339420

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KEYWORDS Alu repeat; T-cell-specific protein.
SOURCE Human peripheral blood (T lymphocyte) cell line AH2, cDNA to mRNA,
ORGANISM clone 228.
REFERENCE Homo sapiens
AUTHORS Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Clayberger, C., Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
TITLE 1 (bases 1 to 1160)
JOURNAL Schall,T.J., Jongstra,J., Dyer,B.J., Jorgensen,J.,
family Davis,M.M. and Krensky,A.M.
MEDLINE A human T cell-specific molecule is a member of a new gene
COMMENT 88285659
provided Draft entry and computer-readable sequence for [1] kindly
FEATURES provided by A.M.Krensky, 24-OCT-1988.
source Location/Qualifiers
CDS 1..1160
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/db_xref="PID:g339421"

/translation="MKVSAARLAVILIATALCAPASAPYSSDTTPCCFAYIARPLPR
AHKEYFYTSGKCSNPVVFVTRKNRQVCANPEKKWVREYINSLEMS"
sig_peptide 27..95
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mat_peptide 96..299
/note="T cell-specific protein"
repeat_region 450..950
/note="Alu-related repeats"
BASE COUNT 298 a 332 c 295 g 235 t
ORIGIN 276 bp upstream of RsaI site.
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121 cctgctgctt tgcctacatt gcccgcacac tgccccgtgc ccacatcaag gagtatttct
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1081 aaggggaggaa cactgcactc ttaagcttcc gccgtctcaa cccctcacag gagttactg
1141 gcaaaatgtca aaaaatgggg
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LOCUS HUMTLI309 520 bp mRNA PRI 14-JAN-1995
DEFINITION Human secreted protein (I-309) mRNA, complete cds.
ACCESSION M57502
NID g339728
KEYWORDS secreted protein.
SOURCE Human T-cell, cDNA to mRNA.
ORGANISM Homo sapiens
REFERENCE Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
AUTHORS Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
TITLE 1 (bases 1 to 520)
JOURNAL Miller,M.D., Hata,S., De Waal Malefyt,R. and Krangel,M.S.
JOURNAL A novel polypeptide secreted by activated human T lymphocytes
J. Immunol. 143 (9), 2907-2916 (1989)

MEDLINE 90038522
 FEATURES Location/Qualifiers
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 /db_xref="GDB:G00-118-872"
 /product="secreted protein I-309"
 /db_xref="PID:g339729"

 /translation="MQIITTAALVCLLLAGMWPEVDVDSKSMQVPPSRCCFSFAEQEIP
 RAILCYRNTSSICSNEGLIFKLKRGKEACALDTVGWVQRHRKMLRHCP SKRK"
 BASE COUNT 140 a 137 c 122 g 121 t
 ORIGIN
 1 accaggctca tcaaagctgc tccaggaagg cccaaaggccag accagaagac atgcagatca
 61 tcaccacagc cctgggtgtc ttgtctgtc ctggatgtg gcccggaaat gtggacagca
 121 agagcatgca ggtacccttc tccatgtttt gtttcttatt tgccggacaa gagattcccc
 181 tggggcaat cctgtttac agaaataccat gtcctatcgat ctccatgtgg ggcttaatata
 241 tcaagctgaa gagaggcaaa gagggctcgcc ctttggacac agttggatgg gttcagaggc
 301 acagaaaaat gctgaggcac tgccctgttccaa aaagaaaaatg agcagatttc tttccattgt
 361 gggctctgga aaccacatgg cttcacatgttcccgaaact accagcccta caccattcc
 421 tctgcccctgc ttttgcgttgg tcacagagga tctgtttgtt cttgataagc tatgttgg
 481 cactttaaac atttaaatta tacaatcatc aaccccccac
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 LOCUS AB000887 687 bp mRNA PRI 05-JUN-1997
 DEFINITION Human mRNA for EBI1-ligand chemokine, complete cds.
 ACCESSION AB000887
 NID 92189952
 KEYWORDS EBI1-ligand chemokine; ELC.
 SOURCE Homo sapiens fetal tissue_lib:lung cDNA to mRNA.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae;
 Homo.
 REFERENCE 1 (bases 1 to 687)
 AUTHORS Yoshida,R., Imai,T., Hieshima,K., Kusuda,J., Baba,M.,
 Kitaura,M.,
 Nishimura,M., Kakizaki,M., Nomiyama,H. and Yoshie,O.
 TITLE Direct Submission
 JOURNAL Submitted (05-FEB-1997) to the DDBJ/EMBL/GenBank databases.
 Hisayuki Nomiyama, Kumamoto University Medical School,
 Department of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan
 (E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:+81-96-373-5063)
 REFERENCE 2 (sites)
 AUTHORS Yoshida,R., Imai,T., Hieshima,K., Kusuda,J., Baba,M.,
 Kitaura,M.,
 Nishimura,M., Kakizaki,M., Nomiyama,H. and Yoshie,O.
 TITLE Molecular cloning of a novel human CC chemokine EBI1-ligand
 chemokine that is a specific functional ligand for EBI1, CCR7
 JOURNAL J. Biol. Chem. 272 (21), 13803-13809 (1997)
 MEDLINE 97298088
 FEATURES Location/Qualifiers
 source 1..687
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /dev_stage="fetal"
 /tissue_lib="lung"
 gene 139..435
 /gene="ELC"

CDS 139..435
 /gene="ELC"
 /note="CC chemokine"
 /codon_start=1
 /product="EBI1-ligand chemokine"
 /db_xref="PID:d1021215"
 /db_xref="PID:g2189953"

 /translation="MALLLALSLLVLWTSPAPTLSGTNDAAEDCCLSVTQKPIPGYIVR
 NFHYLLIKDGCRVPAAVFTTLRGRQLCAPPDQPWVERIIQRLQRTSAKMKRRSS"
 mat_peptide 202..432
 /gene="ELC"
 /product="EBI1-ligand chemokine"
 polyA_signal 657..662
 BASE COUNT 154 a 223 c 173 g 137 t
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 1 cattcccgac ctcacatcac tcacacaccc catttcaccc ctgcacatccca gtcgcctgc
 61 agcctccacac agatccctgca cacacccaga cagtcggcgc tcacacatccca accgttggcc
 121 tgcctctgtt caccctccat ggcctgtca ctgcctctca gcctgtctgtt tctctggact
 181 tccccagccc caactctgag tggcaccat gatgtgaag actgtctgcct gtctgtgacc
 241 cagaaaccca tccctgggta catgtgagg aacttccact accttctcat caaggatggc
 301 tgcagggtgc ctgtctgtt gttcaccaca ctgaggggcc gccagctctg tgcaccccca
 361 gaccagccct gggtagaaacg catcatccag agactgcaga ggacccctcagc caagatgaag
 421 cgcccgagca gttAACCTAT gaccgtgcg aggagccccg gagtccgagt caagcattgt
 481 gaattattac taaacctggg gaaccgagga ccagaaggaa ggaccaggat tccagctct
 541 ctgcaccaga cctgaccaggc caggacaggg cctgggtgt gtgtgagt gatgtgagc
 601 gagagggta gtgtggtcag agtaaagctg ctccacccccc agattgcaat gctaccaata
 661 aagccgcctg gtgtttacaa ctaattt
 //
 LOCUS AB000221 760 bp mRNA PRI 31-JUL-1997
 DEFINITION Homo sapiens mRNA for CC chemokine, complete cds.
 ACCESSION AB000221
 NID g2289718
 KEYWORDS CC chemokine; PARC; pulmonary and activation-regulated chemokine.
 SOURCE Homo sapiens lung cDNA to mRNA.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (bases 1 to 760)
 AUTHORS Nomiyama, H.
 TITLE Direct Submission
 JOURNAL Submitted (04-JAN-1997) to the DDBJ/EMBL/GenBank databases.
 Hisayuki Nomiyama, Kumamoto University Medical School,
 Department of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan
 (E-mail: nomiyama@gpo.kumamoto-u.ac.jp. Tel: 81-96-373-5063,
 Fax: 81-96-372-6140)
 REFERENCE 2 (sites)
 AUTHORS Hieshima, K., Imai, T., Baba, M., Shoudai, K., Ishizuka, K.,
 Nakagawa, T., Tsuruta, J., Takeya, M., Sakaki, Y., Takatsuki, K.,
 Miura, R., Opdenakker, G., Damme, J., Yoshie, O. and Nomiyama, H.
 TITLE A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1alpha/LD78alpha and chemotactic
 JOURNAL for T lymphocytes, but not for monocytes
 MEDLINE J. Immunol. 159 (3), 1140-1149 (1997)
 97376836
 FEATURES Location/Qualifiers
 source 1..760
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /tissue_type="lung"
 gene 64..333
 /gene="PARC"
 CDS 64..333
 /gene="PARC"
 /note="pulmonary and activation-regulated chemokine"

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/codon_start=1
/product="CC chemokine"
/db_xref="PID:d1022520"
/db_xref="PID:g2289719"



LOCUS D86955 799 bp mRNA PRI 06-MAR-1997  

DEFINITION Human mRNA for CC chemokine LARC precursor, complete cds.  

ACCESSION D86955  

NID g1871138  

KEYWORDS CC chemokine LARC precursor.  

SOURCE Homo sapiens cDNA to mRNA.  

ORGANISM Homo sapiens  

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  

Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;  

Hominidae; Homo.  

REFERENCE  

AUTHORS Hieshima,K., Imai,T., Opdenakker,G., Van Damme,J., Kusuda,J.,  

Tei,H., Sakaki,Y., Takatsuki,K., Miura,R., Yoshie,O. and  

Nomiyama,H.  

TITLE Molecular cloning of a novel human CC chemokine liver and  

activation-regulated chemokine (LARC) expressed in liver.  

Chemotactic activity for lymphocytes and gene localization on  

chromosome 2  

JOURNAL J. Biol. Chem. 272 (9), 5846-5853 (1997)  

MEDLINE 97190319  

REFERENCE  

AUTHORS Hieshima,K., Imai,T., Opdenakker,G., Van Damme,J., Kusuda,J.,  

Tei,H., Sakaki,Y., Takatsuki,K., Miura,R., Yoshie,O. and  

Nomiyama,H.  

JOURNAL Unpublished (1996)  

REFERENCE  

AUTHORS Nomiyama,H.  

TITLE Direct Submission  

JOURNAL Submitted (08-AUG-1996) to the DDBJ/EMBL/GenBank databases.  

Hisayuki Nomiyama, Kumamoto University Medical School,  

Department of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan  

(E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:+81-96-373-5063)  

FEATURES  

source Location/Qualifiers  

1..799  

/organism="Homo sapiens"  

/db_xref="taxon:9606"  

/chromosome="2"  

/map="q33-37"  

sig_peptide 59..136  

/gene="LARC"  

CDS 59..349  

/gene="LARC"  

/codon_start=1  

/product="CC chemokine LARC precursor"  

/db_xref="PID:d1013880"  

/db_xref="PID:g1871139"


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LOCUS HUMAR 538 bp mRNA PRI 11-SEP-1996  

DEFINITION Human mRNA for chemokine, complete cds.  

ACCESSION D43767  

NID g1536878  

KEYWORDS chemokine, thymus and activation-regulated; chemokine.  

SOURCE Homo sapiens male peripheral blood cDNA to mRNA, clone:D3A.  

ORGANISM Homo sapiens  

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  

Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;  

Hominidae;  

Homo.  

REFERENCE  

AUTHORS Imai,T., Yoshida,T., Baba,M., Nishimura,M., Kakizaki,M. and  

Yoshie,O.  

TITLE Molecular cloning of a novel T cell-directed CC chemokine  

expressed in thymus by signal sequence trap using Epstein-Barr virus  

vector  

JOURNAL J. Biol. Chem. 271 (35), 21514-21521 (1996)  

MEDLINE 96355526  

REFERENCE  

AUTHORS Imai,T.  

JOURNAL Unpublished (1996)  

REFERENCE  

AUTHORS Imai,T.  

TITLE Direct Submission  

JOURNAL Submitted (07-DEC-1994) to the DDBJ/EMBL/GenBank databases.  

Toshio Imai, Shionogi Institute for Medical Science; 2-5-1 Mishima,  

Settsu, Osaka 566, Japan (Tel:06-382-2612, Fax:06-382-2598)  

FEATURES  

source Location/Qualifiers  

1..538  

/organism="Homo sapiens"  

/db_xref="taxon:9606"  

/clone="D3A"  

/sex="male"  

/tissue_type="peripheral blood"  

CDS 53..337  

/note="thymus and activation regulated"  

/codon_start=1  

/product="chemokine"  

/db_xref="PID:d1008410"  

/db_xref="PID:g1536879"


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BASE COUNT 118 a 168 c 149 g 103 t

ORIGIN

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1 ccctgagcag agggacatgc acacagagac tccctctgg gctctggca ccatggcccc
61 actgaagatg ctggccctgg tcacccctc cctgggggt tctctgcagc acatccacgc
121 agctcgaggg accaatgtgg gccgggagtg ctgcctggag tacttcaagg gagccattcc
181 ccttagaaag ctgaagacgt ggtacccagac atctgaggac tgctccaggatgcctgt
241 tttttaact gtgcaggcga gggccatctg ttcggacccc aacaacaaga gagtgaagaa
301 tgcatgttaaa tacctgcaaa gccttgagag gtcttgaagc ctccctcaccc cagactcctg
361 actgtctccc gggactacct gggacctcca ccgttgggtgt tcaccggccc caccctgagc
421 gcctgggtcc aggggaggcc ttccaggcga aagaagagc cacagtgggg gagatcccat
481 ccccttgtct gaactggcaca aaggggcccat attaaagtct ttatccctc
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LOCUS HUMEOTAXIN 807 bp mRNA PRI 25-SEP-1996

DEFINITION Human mRNA for eotaxin, complete cds.

ACCESSION D49372

NID g1552240

KEYWORDS eotaxin; eosinophil-selective CC chemokine; chemoattractant.

SOURCE Homo sapiens Small intestine, proximal cDNA to mRNA, clone:141.

ORGANISM Homo sapiens

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 807)

AUTHORS Kitaura,M., Nakajima,T., Imai,T., Harada,S., Combadiere,C., Tiffany,H.L., Murphy,P.M. and Yoshie,O.

TITLE Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC chemokine receptor 3

JOURNAL J. Biol. Chem. 271 (13), 7725-7730 (1996)

MEDLINE 96205964

REFERENCE 2 (bases 1 to 807)

AUTHORS Yoshie,O.

TITLE Direct Submission

JOURNAL Submitted (15-FEB-1995) to the DDBJ/EMBL/GenBank databases.

Osamu Yoshie, Shionogi Institute for Medical Science; 2-5-1 Mishima, Settsu, Osaka 566, Japan (E-mail:osamu.yoshie@shionogi.co.jp, Tel:06-382-2612, Fax:06-382-2598)

COMMENT On Sep 20, 1996 this sequence version replaced gi:1313900.

FEATURES Location/Qualifiers

source 1..807
/organism="Homo sapiens"
/db_xref="taxon:9606"
/clone="141"
/tissue_type="Small intestine, proximal"

CDS 99..392
/codon_start=1
/product="eotaxin"
/db_xref="PID:d1008966"
/db_xref="PID:g1552241"

/translation="MKVSAALLWLLIAAAAFSPQGLAGPASVPTCCFNLANRKIPLQRLEYRRITSGKCPQKAVIFKTKLAKDICADPKKKWVQDSMKYLDQKSPTPKP"

misc_signal 548..557
/note="mRNA destabilization signal"

polyA_signal 775..780

polyA_site 807

BASE COUNT 229 a 198 c 147 g 233 t

ORIGIN

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1 gcatttttc aagttttatg atttatttaa cttgtggAAC aaaaataaac cagaaaccac
61 cacctctcac gccaaagtc acacccatcg cctccaaacat gaaggcttcc gcagcacttc
121 tggcgtgtct gtcatgca gtcgtttca gccccccagg gtcgttcc ccagttctg
181 tcccaaccac ctgtgtttt aacctggcca ataggaagat accccatcg cgtactagaga
241 gctacaggag aatcaccagt ggcaatgtc cccagaaagc tgcgttcc aagacccaaac
301 tggccaagga tatctgtgcc gaccccaaga agaagtgggt gcaggattcc atgaagtatc
361 tgacccaaaa atctccaaact cccaaagccat aaataatcac cattttgaa accaaaccag
421 agcctgtggt ttgcctaatt tttttccct ttttacaatg cattttgggg taacccatt
481 atcagtccaa agggcatggg ttttattata tatataata tttttttttt aaaaaaaaaac
541 gtattgcatt taatttatttgg aggctttaaa atttatccctc catgaatatac agttttttt
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601 aaactgtaaa gctttgtca gatttttac cccctggag ccccaattcg atccccgtc
 661 acgtgtggc aatgttcccc ctctcccttc ttccctccctg gaatcttgc aaggccctgg
 721 caaagatgt cagttatgtatgatcattgt tttgtgaac ccaaagtgtg actcattaaa
 781 tggaaatgtaaa ttgttttgc gaaatac

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LOCUS HSCCCHEM 232 bp RNA **PRI** 10-SEP-1996
DEFINITION H.sapiens mRNA for CC-chemokine.
ACCESSION Z69291
NID g1181148
KEYWORDS CC-chemokine.
SOURCE human.
ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 232)
AUTHORS Bartels,J.H., Schlueter,C., Richter,E., Christophers,E. and
 Schroeder,J.M.
TITLE Cloning of a novel human chemokine homologous to human monocyte
 chemoattractant proteins and rodent eotaxins
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 232)
AUTHORS Bartels,J.H.
TITLE Direct Submission
JOURNAL Submitted (01-FEB-1996) Bartels J. H.,
 Christian-Albrechts-Universitaet zu Kiel,
 Dermatology/Hautklinik,
 Mol.Biol.Lab.609, Schittenhelmstr. 7, Kiel, Schleswig-Holstein,
 Germany, D-24105
REFERENCE 3 (bases 1 to 232)
AUTHORS Bartels,J., Schlueter,C., Richter,E., Noso,N., Kulke,R.,
 Christophers,E. and Schroder,J.M.
TITLE Human dermal fibroblasts express eotaxin: molecular cloning,
mRNA expression, and identification of eotaxin sequence variants
JOURNAL Biochem. Biophys. Res. Commun. 225 (3), 1045-1051 (1996)
MEDLINE 96374440
FEATURES Location/Qualifiers
source 1..232
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /clone="clones 4 (9512),
 14 (9512), 15 (9512), 10 (9601), 11 (9601)"
 /tissue_type="foreskin"
 /cell_type="fibroblast"
 /sex="Male"
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 /citation=[1]
 /product="CC-chemokine"
mRNA 56..109
 /citation=[1]
sig_peptide 56..109
 /citation=[1]
CDS 56..>232
 /function="putative chemoattractant protein"
 /note="sequence homology to human MCP-1, MCP-2 and
 MCP-3
 and to rodent eotaxins"
 /citation=[1]
 /codon_start=1
 /product="CC-chemokine, preprotein"
 /db_xref="PID:e221070"
 /db_xref="PID:g1181149"
 /db_xref="SWISS-PROT:P50877"

/translation="MKVSAALLWLLLIAAAFPSPQGLAGPASVPTTCCFNLANRKIPLQ
RLESYRRITSGKCPQ"
mat_peptide 110..>232
 /citation=[1]
 /function="putative chemoattractant protein"
 /product="CC-chemokine"
BASE COUNT 55 a 82 c 50 g 42 t 3 others
ORIGIN 1 accaaaccag aaaccwccam ytctcacgcc aaagctcaca cttcagcct ccaacatgaa

61 ggtctccgca gcgcttctgt ggctgctgct catagcggt gccttcagcc cccagggct
 121 cgctgggcca gcttctgtcc caaccacctg ctgctttaac ctggccaata ggaagatacc
 .. 181 cttcagcga ctagagagct acaggagaat caccagtggc aatgtcccc ag
 //

LOCUS HSHCC1GEN 4037 bp DNA **PRI** 01-OCT-1995
DEFINITION H.sapiens gene for chemokine HCC-1.
ACCESSION Z49269
NID g1004266
KEYWORDS chemokine.
SOURCE human.
ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 4037)
AUTHORS Pardigol,A., Maegert,H.J., Cieslak,A., Hill,O., Schulz-Knappe,P.
 and Forssmann,W.G.
TITLE Nucleotide Sequence of the Gene for the Human Chemokine HCC-1
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 4037)
AUTHORS Pardigol,A.
TITLE Direct Submission
JOURNAL Submitted (18-MAY-1995) Andreas Pardigol, Molecular Biology,
Lower Saxony Institute for Peptide Research, Feodor-Lynen-Strasse 31,
 Hannover, Lower Saxon, 30625, Germany
FEATURES
 source Location/Qualifiers
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 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /clone="ph3b7"
 /dev_stage="adult"
 /tissue_type="placenta"
 /clone_lib="lambda FIX II, Cat.Nr. 946203, Stratagene"
 /sex="male"
 TATA_signal 727..733
 /note="putative, determined by consensus rules."
 5'UTR 764..833
 /note="first base determined by means of consensus
 rules"
 exon 764..912
 /note="first base determined by means of consensus
 rules;
 base 780 is the first base of cDNA (Z49270)"
 CDS /number=1
 join(834..912,3021..3135,3585..3672)
 /codon_start=1
 /product="chemokine HCC-1"
 /db_xref="PID:g1004267"

/translation="MKISVAAIPFFLLITIALGTKTESSSRGPYHPSECCFTYTTYKI
 PRQRIMDYYETNSQCSKPGIVFITKRGHSVCTNPSDKWVQDYIKDMKEN"
 intron 913..3020
 /number=1
 exon 3021..3135
 /number=2
 intron 3136..3584
 /number=2
 exon 3585..3817
 /number=3
 3'UTR 3673..3817
BASE COUNT 1023 a 1048 c 1004 g 962 t
ORIGIN
 1 gagctccgtt gggagtcaca tggttttta tggcataatg ggtgagaaca cagacttgg
 61 agccaaacca cctgaattt aaccccaagg ccatttacca actgtcaaaa gcttaggctt
 121 tgattctaag cctgtttcct caactgtgt tctaaagatt aaataggcta atattcataa
 181 ggcaactggg acagtggctt gtgtgtatag caaccattat ataagtgaat tatctactga
 241 gcaccacagc acttcttccac tccatgggtt ggtgaccaga atggagatga gacagagaac
 301 tgcagggtctt gcttcggat taaatggat tttcccttgc ccaatggac ctgacttgg
 361 ggagtcttgg cctcatttca ttacccaaa caccctctag tctctatgt aacagatcct
 421 gaatgtccag gccccacgtg gcctgttcta aggctgaga tggaaatttga tacaggacac

//
LOCUS HSCC21 925 bp mRNA PRI 30-JUN-1998
DEFINITION H.sapiens mRNA for chemokine CC-2 and CC-1.
ACCESSION Z70292
NID g1296608
KEYWORDS chemokine CC-1; chemokine CC-2.
SOURCE human.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 925)

AUTHORS Pardigol,A., Forssmann,U., Zucht,H.D., Loetscher,P.,
TITLE Schulz-Knappe,P., Baggiolini,M., Forssmann,W.G. and Magert,H.J.
and HCC-2, a human chemokine: gene structure, expression pattern,
biological activity
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 95 (11), 6308-6313 (1998)
MEDLINE 98263352
REFERENCE 2 (bases 1 to 925)
AUTHORS Pardigol,A.
TITLE Direct Submission
JOURNAL Submitted (25-MAR-1996) Andreas Pardigol, IV - Molecular
Biology.
Lower Saxony Institute for Peptide Research, Feodor-Lynen-
Strasse 31, Hannover, Lower Saxony, 30625, Germany
FEATURES Location/Qualifiers
source 1..925
/organism="Homo sapiens"
/db_xref="taxon:9606"
/dev_stage="adult"
/tissue_type="liver"
/clone_lib="PCR fragments"
5'UTR 1..55
CDS 56..397
/note="putative; first coding region of a bicistronic
mRNA"
/codon_start=1
/product="chemokine CC-2"
/db_xref="PID:e233855"
/db_xref="PID:g1296609"
/db_xref="SWISS-PROT:Q16663"

/translation="MKVSVAALSLMLVAVLGSQAQFTNDAETELMMSKLPLENPVVL
NSFHFAADCCTS YISQSIPCSLMSYFETSSCECSKPGVIFLTKKGRQVCAKPSGPGVQ
DCMKKLKPYSI"
misc_feature 398..498
/note="spacing region between two coding regions of
the
bicistronic mRNA"
CDS 499..780
/codon_start=1
/evidence=experimental
/product="chemokine CC-1"
/db_xref="PID:e233856"
/db_xref="PID:g1296610"
/db_xref="SWISS-PROT:Q16627"

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PRQRIMDYETNSQCSKPGIVFITKRGHSVCTNPSDKWVQDYIKDMKEN"
3'UTR 781..925
polyA_signal 902..908
BASE COUNT 240 a 296 c 199 g 190 t
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1 ccaggaagca gtgagccag gagtcctcg ccagccctgc ctgcccacca ggaggatgaa
61 ggtctccgtg gtcgcctct cctgcctcat gcttggat gtcctggat cccaggccca
121 gttcacaaat gatcgagaga cagatataat gatgtcaaaat cttccactgg aaaatccagt
181 agttctgaac agcttcaact ttgtctgtga ctgctgcacc tcctacatct cacaaggcat
241 cccgtgttca ctcatggaaa gttatgttga aacgagcagc gagtgctcca agccagggt
301 catattccctc accaagaagg ggcggcaagt ctgtgcacaa cccagtggtc cgggagttca
361 ggattgcatg aaaaagctga agccctactc aatataataa taaagagaca aaagaggccca
421 gcaacccacc tccaaacacct cctgagccctc tgaagctccc accaggccag ctctccccc
481 acaacagctt cccacagcat gaagatctcc gtggctgcac ttcccttctt cctccctcatc
541 accatcgccc tagggaccaa gactaatcc tcctcacggg gaccttacca cccctcagag
601 tgctgcttca cttacactac ctacaaatcc cccgtgtcagc ggattatggta ttactatgag
661 accaacagcc agtgctccaa gcccggaaatt gtcttcatca caaaaagggg ccattccgtc
721 tgtaccaacc ccagtgcacaa gtgggtccag gactatataa aggacatgaa ggagaactga
781 gtgacccaga agggggtggcg aaggcacagc tcagagacat aaagagaaga tgccaaaggcc
841 ccctccttcca cccaccgcata actctcagcc ccagtccaccc tcttggagct tccctgctt
901 gaattaaaga ccactcatgc tttc
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LOCUS HSCC23 973 bp RNA PRI 03-MAY-1996
 DEFINITION H.sapiens mRNA for chemokine CC-2 and CC-3.
 ACCESSION Z70293
 NID g1296611
 KEYWORDS Human chemokine CC-2; Human chemokine CC-3.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (bases 1 to 973)
 REFERENCE Pardigol, A., Maegert, H.J., Zucht, H.D., Forssmann, W.G. and
 AUTHORS Schulz-Knappe, P.
 TITLE Transcription of a Human Tandem Gene results in a Mature
 Bicistronic mRNA encoding two Novel CC-Chemokines
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 973)
 AUTHORS Pardigol, A.
 TITLE Direct Submission
 JOURNAL Submitted (25-MAR-1996) Andreas Pardigol, IV - Molecular
 Biology.
 Strasse Lower Saxony Institute for Peptide Research, Feodor-Lynen-
 31. Hannover, Lower Saxony, 30625, Germany
 FEATURES Location/Qualifiers
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 /db_xref="PID:g1296613"

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 3'UTR 829..973
 polyA_signal 950..956
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 181 agttctgaac agctttact ttgctgtga ctgctgcacc tcctacatct cacaaggat
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 301 catattcctc accaagaagg ggcggcaagt ctgtccaaa cccagtggc cgggagttca
 361 ggatgtcatg aaaaagctga agccctactc aatataataa taaagagaca aaagaggcca
 421 gccacccacc tccaacaccc cctgagcctc tgaagctccc accaggccag ctctccccc

AUTHORS Pardigol,A., Forssmann,U., Zucht,H.D., Loetscher,P.,
 Schulz-Knappe,P., Baggolini,M., Forssmann,W.G. and Magert,H.J.
 TITLE and HCC-2, a human chemokine: gene structure, expression pattern,
 biological activity
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 95 (11), 6308-6313 (1998)
 MEDLINE 98263352
 REFERENCE 2 (bases 1 to 925)
 AUTHORS Pardigol,A.
 TITLE Direct Submission
 JOURNAL Submitted (25-MAR-1996) Andreas Pardigol, IV - Molecular
 Biology, Lower Saxony Institute for Peptide Research, Feodor-Lynen-
 Strasse 31, Hannover, Lower Saxony, 30625, Germany
 FEATURES Location/Qualifiers
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 CDS 499..780
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 /evidence=experimental
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 /db_xref="PID:e233856"
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 /db_xref="SWISS-PROT:Q16627"

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 polyA_signal 902..908
 BASE COUNT 240 a 296 c 199 g 190 t
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 121 gttcacaaat gatgcagaga cagatgtt gatgtcaaag cttccactgg aaaatccagt
 181 agttctgaac agctttcaact ttgctgctga ctgctgcacc tcctacatct cacaaggcat
 241 cccgtgttca ctcataaaaa gttatgttga aacgagcagc gagtgctcca agccaggtgt
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 601 tgctgcttca cttacactac ctacaagatc cccggtcagc ggattatggta ttactatgag
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1141 ttttcataag gaagtccgga tggaaatatt cacattaatc attttgcag agactttgct
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 1261 aaaaaaaaaa aaagagagag agagagaaga agaagaagaa gagacacaaa tctctacctc
 1321 ccatgttaag ctttcagga caggaaaga aagggtatga gacacggcta ggggtaaact
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 LOCUS AB007454 1503 bp mRNA PRI 09-APR-1998
 DEFINITION Homo sapiens mRNA for chemokine LEC precursor, complete cds.
 ACCESSION AB007454
 NID 92723285
 KEYWORDS chemokine LEC precursor.
 SOURCE Homo sapiens liver cDNA to mRNA.
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (sites)
 AUTHORS Shoudai,K., Hieshima,K., Fukuda,S., Iio,M., Miura,R., Imai,T.,
 Yoshie,O. and Nomiyama,H.
 TITLE Isolation of cDNA encoding a novel human CC chemokine NCC-4/LEC
 JOURNAL Biochim. Biophys. Acta 1396 (3), 273-277 (1998)
 MEDLINE 98207719
 REFERENCE 2 (bases 1 to 1503)
 AUTHORS Nomiyama,H.
 TITLE Direct Submission
 JOURNAL Submitted (19-SEP-1997) to the DDBJ/EMBL/GenBank databases.
 Hisayuki Nomiyama, Kumamoto University Medical School,
 Department of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860-0811,
 Japan (E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:81-96-373-5063,
 Fax:81-96-372-6140)
 FEATURES Location/Qualifiers
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 /db_xref="PID:g2723286"

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 mat_peptide 146..436
 polyA_signal 560..565
 polyA_signal 1485..1490
 BASE COUNT 417 a 374 c 312 g 400 t
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 121 tatcattact tcggcttctc gcagccagcc aaaagtccct gagtgggtga acacccatc
 181 cacctgtgc ctgaagttt atgaaaagt gttgccaagg agactagtgg tgggatacag
 241 aaaggccctc aactgtcacc tgccagcaat catcttcgtc accaagagga accgagaagt
 301 ctgcaccaac cccaatgacg actgggtcca agatcatac aaggatccca acctacctt
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 841 gggtggcgaa ggcacagctc agagacataa agagaagatg ccaaggcccc ctccctccacc
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 961 actcatgctc ttc
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LOCUS HSU91746 1430 bp mRNA **PRI** 12-MAR-1998
DEFINITION Homo sapiens IL-10-inducible chemokine (HCC-4) mRNA, complete
cds.
ACCESSION U91746
NID g2581780
KEYWORDS .
SOURCE human.
ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1430)
AUTHORS Hedrick, J.A., Helms, A., Gorman, D. and Zlotnik, A.
TITLE Identification of a novel human CC chemokine upregulated by IL-
 10
JOURNAL Blood (1998) In press
REFERENCE 2 (bases 1 to 1430)
AUTHORS Hedrick, J.A., Helms, A., Gorman, D. and Zlotnik, A.
TITLE Direct Submission
JOURNAL Submitted (02-MAR-1997) Immunology, DNAX Research Institute,
 901 California Ave, Palo Alto, CA 94304, USA
FEATURES
source Location/Qualifiers
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CDS 1..363
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BASE COUNT 401 a 351 c 293 g 385 t
ORIGIN

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    961 tcacaccatg acacaaggca ttcttataac ttggccagg tccagccctc cagatgtatc
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Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
 Hominidae; Homo.
 REFERENCE 1 (bases 1 to 821)
 AUTHORS Hromas,R., Gray,P.W., Chantry,D., Godiska,R., Krathwohl,M.,
 Fife,K., Bell,G.I., Takeda,J., Aronica,S., Gordon,M.,
 Cooper,S., Broxmeyer,H.E. and Klemsz,M.J.
 TITLE Cloning and characterization of exodus, a novel beta-chemokine
 JOURNAL Blood 89 (9), 3315-3322 (1997)
 MEDLINE 97275143
 REFERENCE 2 (bases 1 to 821)
 AUTHORS Hromas,R.A.
 TITLE Direct Submission
 JOURNAL Submitted (17-JUL-1996) Indiana University Medical Center,
 Medicine, 975 W. Walnut St., Indianapolis, IN 46202, USA
 FEATURES Location/Qualifiers
 source 1..821
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 /dev_stage="adult"
 CDS 43..330
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 121 agcaactttg actgctgtct tggatcacaca gaccgtattc ttcatcttatttttgc
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 LOCUS HSU88320 828 bp mRNA PRI 18-DEC-1997
 DEFINITION Human beta chemokine Exodus-2 mRNA, complete cds.
 ACCESSION U88320
 NID g2196919
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 828)
 AUTHORS Hromas,R., Kim,C.H., Klemsz,M., Krathwohl,M., Fife,K.,
 Cooper,S.,

TITLE Schnizlein-Bick, C. and Broxmeyer, H.E.
 chemokine Isolation and characterization of Exodus-2, a novel C-C
 with a unique 37-amino acid carboxyl-terminal extension
 JOURNAL J. Immunol. 159 (6), 2554-2558 (1997)
 MEDLINE 97444139
 REFERENCE 2 (bases 1 to 828)
 AUTHORS Hromas, R.A.
 TITLE Direct Submission
 JOURNAL Submitted (04-FEB-1997) Medicine, Indiana University Medical
 Center, 975 West Walnut, Indianapolis, IN 46202, USA
 FEATURES Location/Qualifiers
 source 1..828
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 /note="PCR amplified from activated THP-1 cells"
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 781 aataaaaagc ttttctgtct tccccacccaa aaaaaaaaaa aaaaaaaaaa
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 LOCUS HSU88321 502 bp mRNA PRI 22-JUN-1998
 DEFINITION Human beta chemokine Exodus-3 mRNA, complete cds.
 ACCESSION U88321
 NID g2196921
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 502)
 AUTHORS Hromas, R.A., Gray, P., Klemsz, M., Fife, K. and Broxmeyer, H.
 TITLE DCCL chemokines represent a novel beta chemokine subfamily
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 502)
 AUTHORS Hromas, R.A.
 TITLE Direct Submission
 JOURNAL Submitted (04-FEB-1997) Medicine, Indiana University Medical
 Center, 975 West Walnut, Indianapolis, IN 46202, USA
 REFERENCE 3 (bases 1 to 502)
 AUTHORS Hromas, R.A.
 TITLE Direct Submission
 JOURNAL Submitted (22-JUN-1998) Medicine, Indiana University Medical
 Center, 975 West Walnut, Indianapolis, IN 46202, USA
 REMARK Amino acid sequence updated by submitter
 FEATURES Location/Qualifiers
 source 1..502

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/organism="Homo sapiens"
/note="PCR amplified from THP-1 cells"
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/dev_stage="adult"
CDS
120..416
/note="Mip-3alpha/ELC/CKbeta1"
/codon_start=1
/product="beta chemokine Exodus-3"
/db_xref="PID:g3243080"


HSU86358 879 bp mRNA PRI 11-SEP-1997
Human chemokine (TECK) mRNA, complete cds.
U86358
g2388626
KEYWORDS
SOURCE
ORGANISM
REFERENCE
AUTHORS
JOURNAL
FEATURES
source
gene
CDS
human.
Homo sapiens
Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
1 (bases 1 to 879)
Vicari,A.P., Figueiroa,D.J., Hedrick,J.A., Foster,J.S., Singh,K.P., Menon,S., Copeland,N.G., Gilbert,D.J., Jenkins,N.A., Bacon,K.B. and Ziotnik,A.
TECK: a novel cc chemokine specifically expressed by thymic dendritic cells and potentially involved in T cell development
Immunology 7, 291-301 (1997)
2 (bases 1 to 879)
Vicari,A.P. and Ziotnik,A.
Direct Submission
Submitted (21-JAN-1997) Immunology, DNAX Research Institute, 901 California Ave., Palo Alto, CA 94304, USA
Location/Qualifiers
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ORIGIN

1 atgaacacctgt ggctcctggc ctgcctggc gcccggctcc tgggagcctg gccccccgc
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11

LOCUS AB002409 852 bp mRNA **PRI** 15-AUG-1997
DEFINITION Homo sapiens mRNA for SLC, complete cds.
ACCESSION AB002409
NID g2335034
KEYWORDS SLC; mature ELC.
SOURCE Homo sapiens cDNA to mRNA.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (bases 1 to 852)
AUTHORS Nomiyama, H.
TITLE Direct Submission
JOURNAL Submitted (28-MAR-1997) to the DDBJ/EMBL/GenBank databases.
Department Hisayuki Nomiyama, Kumamoto University Medical School,
of Biochemistry; Honjo 2-2-1, Kumamoto, Kumamoto 860, Japan
(E-mail:nomiyama@gpo.kumamoto-u.ac.jp, Tel:81-96-373-5063,
Fax:81-96-372-6140)
REFERENCE 2 (bases 1 to 852)
AUTHORS Nagira, M., Imai, T., Hieshima, K., Kusuda, J., Ridanpaa, M.,
Takagi, S.,
TITLE Nishimura, M., Kakizaki, M., Nomiyama, H. and Yoshie, O.
Molecular Cloning of a Novel Human CC Chemokine Secondary
Lymphoid-Tissue Chemokine (SLC) That is an Efficient
Chemoattractant for Lymphocytes and Mapped to Chromosome 9p13
JOURNAL Unpublished (1997)
FEATURES Location/Qualifiers
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BASE COUNT 205 a 279 c 217 g 151 t
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ORIGIN

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 841 cttcccaccc gc
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 Locus AF055467 1481 bp mRNA PRI 06-AUG-1998
 Definition Homo sapiens monotactin-1 mRNA, complete cds.
 Accession AF055467
 NID g3395775
 Keywords
 Source human.
 Organism Homo sapiens
 Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 Reference 1 (bases 1 to 1481)
 Authors Youn, B.S., Zhang, S., Broxmeyer, H.E., Antol, K., Fraser, M.J. Jr.,
 Hangoc, G. and Kwon, B.S.
 Title Isolation and characterization of LMC, a novel lymphocyte and
 monocyte chemoattractant human CC chemokine, with
 myelosuppressive
 activity
 Journal Biochem. Biophys. Res. Commun. 247 (2), 217-222 (1998)
 MEDLINE 98308096
 Reference 2 (bases 1 to 1481)
 Authors Youn, B.S. and Kwon, B.S.
 Title Direct Submission
 Journal Submitted (24-MAR-1998) Microbiology and Immunology, Indiana
 University, School of Medicine, 605 Barnhill Dr. Medical
 Science Bldg., Indianapolis, IN 46202, USA
 Features Location/Qualifiers
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 5'UTR 1..34
 CDS 35..397
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 3'UTR 398..1481
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1321 aagaagaaga agaagagaca caaatctcta cctccatgt taagcttgc agacaggga
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1441 aataaaaactc ctttatttga caaaaaaaaaaaa aaaaaaaaaaaa a

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LOCUS HSRNAATAC 557 bp RNA **PRI** 06-JUL-1995
DEFINITION H.sapiens mRNA for ATAC protein.
ACCESSION X86474
NID g895846
KEYWORDS ATAC gene.
SOURCE human.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 557)
AUTHORS Muller,S., Dorner,B., Korthauer,U., Mages,H.W., D'Apuzzo,M.,
Senger,G. and Kroczek,R.A.
TITLE Cloning of ATAC, an activation-induced, chemokine-related
molecule exclusively expressed in CD8+ T lymphocytes
JOURNAL Eur. J. Immunol. 25 (6), 1744-1748 (1995)
MEDLINE 95339892
REFERENCE 2 (bases 1 to 557)
AUTHORS Kroczek,R.A.
TITLE Direct Submission
JOURNAL Submitted (20-APR-1995) R.A. Kroczek, Molecular Immunology,
Robert-Koch-Institute, Nordufer 20, 13353 Berlin, FRG
FEATURES Location/Qualifiers
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CDS 25..369
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polyA_signal 469..474
polyA_signal 534..539
BASE COUNT 157 a 139 c 112 g 149 t
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1 gcacagctca gcaggaccc agccatgaga cttctcatcc tggccctctt tggcatctgc
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301 aacatgatcc agaccaagcc aacaggaacc cagcaatcga ccaatacagc tttgactctg
361 actggctagt agtctctggc accctgtccg tctccagcca gccagctcat ttcaactttac
421 acgctcatgg actgagttt tactcgcctt ttatgaaagc actgcatgaa taaaattatt
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541 tattttat taagaat
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LOCUS HSU85767 563 bp mRNA PRI 01-APR-1997
 DEFINITION Human myeloid progenitor inhibitory factor-1 MPIF-1 mRNA,
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 ACCESSION U85767
 NID g1916249
 KEYWORDS .
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (bases 1 to 563)
 REFERENCE
 AUTHORS Patel, V.P., Kreider, B.L., Li, Y., Li, H., Leung, K., Salcedo, T.,
 Nardelli, B., Pippalla, V., Gentz, S., Thotakura, R., Parmelee, D.,
 Gentz, R. and Garotta, G.
 TITLE Molecular and functional characterization of two novel human C-
 C
 chemokines as inhibitors of two distinct classes of myeloid
 progenitors
 JOURNAL J. Exp. Med. (1997) In press
 2 (bases 1 to 563)
 AUTHORS Li, H. and Patel, V.P.
 TITLE Direct Submission
 JOURNAL Submitted (17-JAN-1997) Cell Biology, Human Genome Sciences,
 9410 Keywest Ave., Rockville, MD 20850, USA
 FEATURES Location/Qualifiers
 source 1..563
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 /db_xref="taxon:9606"
 CDS 31..393
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 361 ctggacacac ggatcaagac caggaagaat tgaacttgta aaggtgaagg gacacaagtt
 421 gccagccacc aactttcttg cctcaactac cttcctgaaat tattttttta agaagcattt
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 541 aaaaaaaaaa aaaaaaaaaa aaa
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LOCUS HSU85768 360 bp mRNA PRI 01-APR-1997
 DEFINITION Human myeloid progenitor inhibitory factor-1 MPIF-2 mRNA,
 complete
 cds.
 ACCESSION U85768
 NID g1916251
 KEYWORDS .
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (bases 1 to 360)
 REFERENCE
 AUTHORS Patel, V.P., Kreider, B.L., Li, Y., Li, H., Leung, K., Salcedo, T.,
 Nardelli, B., Pippalla, V., Gentz, S., Thotakura, R., Parmelee, D.,
 Gentz, R. and Garotta, G.
 TITLE Molecular and functional characterization of two novel human C-

C

chemokines as inhibitors of two distinct classes of myeloid progenitors
 JOURNAL J. Exp. Med. (1997) In press
 REFERENCE 2 (bases 1 to 360)
 AUTHORS Li, H. and Patel, V.P.
 TITLE Direct Submission
 JOURNAL Submitted (17-JAN-1997) Cell Biology, Human Genome Sciences,
 9410 Keywest Ave., Rockville, MD 20850, USA

FEATURES Location/Qualifiers
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BASE COUNT 85 a 106 c 96 g 73 t
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LOCUS HUMSDF1A 1847 bp mRNA PRI 26-DEC-1996
 DEFINITION Human pre-B cell stimulating factor homologue (SDF1a) mRNA, complete cds.
 ACCESSION L36034
 NID g1220363
 KEYWORDS intercrine; intercrine CXC subfamily; pre-B cell stimulating factor homologue; alpha-chemokine.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 1847)
 AUTHORS Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinohara, T. and Honjo, T.
 TITLE Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene
 JOURNAL Genomics 28 (3), 495-500 (1995)
 MEDLINE 96039262
 FEATURES Location/Qualifiers
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 CDS 80..349
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LOCUS      HUMSDF1B      3524 bp      mRNA      PRI      26-DEC-1996
DEFINITION Human pre-B cell stimulating factor homologue (SDF1b) mRNA,
complete cds.
ACCESSION  L36033
NID        g1220365
KEYWORDS   intercrine; intercrine CXC subfamily; pre-B cell stimulating
factor
SOURCE      human.
ORGANISM   Homo sapiens
             Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
             Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 3524)
AUTHORS   Shirozu,M., Nakano,T., Inazawa,J., Tashiro,K., Tada,H.,
Shinohara,T. and Honjo,T.
TITLE      Structure and chromosomal localization of the human stromal
cell-derived factor 1 (SDF1) gene
JOURNAL    Genomics 28 (3), 495-500 (1995)
MEDLINE    96039262
FEATURES   Location/Qualifiers
source      1..3524
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CDS        80..361
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            /db_xref="PID:g1220366"

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gene 80..358
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 BASE COUNT 903 a 886 c 793 g 942 t
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 ACCESSION AJ002211

NID g2832410
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 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 663)
 AUTHORS Legler,D.F., Loetscher,M., Roos,R.S., Clark-Lewis,I.,
 Bagliolini,M.
 and Moser,B.
 TITLE B cell-attracting chemokine 1, a human CXC chemokine expressed
 in lymphoid tissues, selectively attracts B lymphocytes via
 BLR1/CXCR5
 JOURNAL J. Exp. Med. 187 (4), 655-660 (1998)
 MEDLINE 98130629
 REFERENCE 2 (bases 1 to 663)
 AUTHORS Moser,B.
 TITLE Direct Submission
 JOURNAL Submitted (05-NOV-1997) Moser B., University of Bern, Theodor
 Kocher Institute, Freiestrasse 1, CH-3012 Bern, SWITZERLAND
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 LOCUS HSHUMIG 2545 bp RNA PRI 16-NOV-1993
 DEFINITION H.sapiens Humig mRNA.
 ACCESSION X72755 S60728
 NID g311375
 KEYWORDS chemokine; cytokine; Humig gene; secreted protein.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 2545)
 AUTHORS Farber,J.M.
 TITLE Direct Submission
 JOURNAL Submitted (22-MAR-1993) J.M. Farber, Johns Hopkins Univ. School

of Medicine, Ross 1147, 720 Rutland Avenue, Baltimore, MD 21205,

2161 ctttccaaa ttgaatcaact gctcacactg ctgatgattt agagtgtgtt ccgggtggaga
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LOCUS HSHUMIG 2545 bp RNA PRI 16-NOV-1993
 DEFINITION H. sapiens Humig mRNA.
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 NID g311375
 KEYWORDS chemokine; cytokine; Humig gene; secreted protein.
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 2545)
 AUTHORS Farber, J.M.
 TITLE Direct Submission
 JOURNAL Submitted (22-MAR-1993) J.M. Farber, Johns Hopkins Univ. School
 of Medicine, Ross 1147, 720 Rutland Avenue, Baltimore, MD 21205,
 USA
 REFERENCE 2 (bases 1 to 2545)
 AUTHORS Farber, J.M.
 TITLE HuMic: a new human member of the chemokine family of cytokines
 JOURNAL Biochem. Biophys. Res. Commun. 192 (1), 223-230 (1993)
 MEDLINE 93236577
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LOCUS      AF002985      995 bp      mRNA      PRI      01-NOV-1997
DEFINITION  Homo sapiens putative alpha chemokine (H174) mRNA, complete
cds.
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NID        g2580585
KEYWORDS
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ORGANISM  Homo sapiens
Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 995)
AUTHORS   Jacobs, K.A., Collins-Racie, L.A., Colbert, M., Duckett, M.,
Golden-Fleet, M., Kelleher, K., Kriz, R., LaVallie, E.R.,
Merberg, D., Spaulding, V., Stover, J., Williamson, M.J. and McCoy, J.M.
TITLE     A genetic selection for isolating cDNAs encoding secreted
proteins
JOURNAL   Gene 198 (1-2), 289-296 (1997)
MEDLINE   98036061
REFERENCE  2 (bases 1 to 995)
AUTHORS   Jacobs, K.A., Collins-Racie, L.A., Colbert, M., Duckett, M.,
Golden-Fleet, M., Kelleher, K., Kriz, R., LaVallie, E.R.,
Merberg, D., Spaulding, V., Stover, J., Williamson, M.J. and McCoy, J.M.
TITLE     Direct Submission
JOURNAL   Submitted (07-MAY-1997) Genetics Institute, 87 Cambridge Park
Drive, Cambridge, MA 02140, USA
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LOCUS AF030514 1371 bp mRNA PRI 17-JUN-1998
DEFINITION Homo sapiens interferon stimulated T-cell alpha chemoattractant precursor, mRNA, complete cds.
ACCESSION AF030514
NID g3219692
KEYWORDS
SOURCE
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1371)
AUTHORS Cole, K.E., Strick, C.A., Paradis, T.J., Ogborne, K.T.,
Loetscher, M., Gladue, R.P., Lin, W., Boyd, J.G., Moser, B., Wood, D.E.,
Sahagan, B.G.
and Neote, K.
TITLE Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3
J. Exp. Med. 187 (12), 2009-2021 (1998)
JOURNAL
MEDLINE 98290735
REFERENCE 2 (bases 1 to 1371)
AUTHORS Cole, K.E., Strick, C.A. and Sahagan, B.G.
TITLE Direct Submission
JOURNAL Submitted (20-OCT-1997) Molecular Sciences, Pfizer, Inc.,
Eastern
FEATURES Point Road, Groton, CT 06340, USA
source Location/Qualifiers
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961	atgttagggaa	acattcttat	gcatcatttg	gttgtttta	taaccaattt	attaaatgt				
1021	attcataaaa	tgtactatga	aaaaaaattat	acgttatggg	atactggcaa	cagtgcacat				
1081	atttccataac	caaaatttagca	gcacccgtct	taatttgcatt	tttttcaact	tttatttcatt				
1141	gagatgtttt	gaagcaatta	ggatatgtgt	gtttactgtt	ctttttgttt	tgatccgttt				
1201	gtataaaatga	tagcaatata	ttggacacat	ttgaaataca	aatgttttt	gtctaccaaa				
1261	aaaaaatgtt	aaaaataaag	caaatgtata	cctagcaatc	acttttaccc	tttgcatttc				
1321	tgtctttag	aaaaatacat	aatctaatac	aaaaaaaaaa	aaaaaaaaaa	a				

LOCUS AF030514 1371 bp mRNA PRI 17-JUN-1998
 DEFINITION Homo sapiens interferon stimulated T-cell alpha chemoattractant precursor, mRNA, complete cds.
 ACCESSION AF030514
 NID g3219692
 KEYWORDS
 SOURCE human.
 ORGANISM Homo sapiens
 Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
 Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 1371)
 AUTHORS Cole,K.E., Strick,C.A., Paradis,T.J., Ogborne,K.T.,
 Loetscher,M., Gladue,R.P., Lin,W., Boyd,J.G., Moser,B., Wood,D.E.,
 Sahagan,B.G.
 and Neote,K.
 TITLE Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3
 JOURNAL J. Exp. Med. 187 (12), 2009-2021 (1998)
 MEDLINE 98290735
 REFERENCE 2 (bases 1 to 1371)
 AUTHORS Cole,K.E., Strick,C.A. and Sahagan,B.G.
 TITLE Direct Submission
 JOURNAL Submitted (20-OCT-1997) Molecular Sciences, Pfizer, Inc.,
 Eastern Point Road, Groton, CT 06340, USA
 FEATURES Location/Qualifiers
 source 1..1371
 /organism="Homo sapiens"
 /db_xref="taxon:9606"
 /chromosome="4"
 /cell_type="astrocytes"
 sig_peptide 70..132
 70..354
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 /codon_start=1
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 /db_xref="PID:g3219693"
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  /evidence=not_experimental
  /product="interferon stimulated T-cell alpha
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  61  aaaacaaaaca  tgagtgtcaa  gggcatggct  atagccttgg  ctgtgatatt  gtgtgctaca
  121 gttgttcaag  gcttcccccatt  gttccaaaaga  ggacgctgtc  tttgcataagg  ccctggggta
  181 aaagcagtga  aagtggcaga  tattgagaaa  gcctccataa  tgtacccaag  taacaactgt
  241 gacaaaatag  aagtgattat  taccctgaaa  gaaaaataag  gacaacgatg  cctaaatccc
  301 aaatcgaagc  aagcaaggct  tataatcaaa  aaagttgaaa  gaaaagaattt  taaaaatata
  361 caaaacatata  gaagtcctgg  aaaagggcat  ctgaaaaacc  tagaacaagt  ttaactgtga
  421 ctactgaaat  gacaagaatt  ctacagttagg  aaactgagac  ttttctatgg  ttttgtact
  481 ttcaactttt  gtacagttagt  gtgaaggatg  aaaggtgggt  gaaaggacca  aaaacagaaaa
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  601 aatggatttc  tagggaaaac  tacccataga  aaggctgggt  accatccggag  ttacaaaagt
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  841 cattattact  ggagtcaagc  ccttataagt  caaaagcattc  tatgtgtcg  aaagcattcc
  901 tcaaaacattt  tttcatgca  atacacaytt  ctttccccca  atatcatgt  gcacatcaat
  961 atgtaggaaa  acattcttt  gcatcattt  gtttgggtt  taaccaattt  attaaatgt
  1021 attccataaaa  tttttttttt  ttatgtatgt  aaaaaattttt  acgtctatgg  atactggca  cagtgcacat
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  1261 gaaaaatgtt  gaaaaataaag  caaatgtata  cctagcaatc  acttttactt  tttgtatattc
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  LOCUS      AF030514      1371 bp      mRNA      PRI      17-JUN-1998
  DEFINITION  Homo sapiens interferon stimulated T-cell alpha chemoattractant
  precursor, mRNA, complete cds.
  ACCESSION   AF030514
  NID         g3219692
  KEYWORDS
  SOURCE
  ORGANISM   Homo sapiens
  Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
  Primates; Catarrhini; Hominidae; Homo.
  REFERENCE   1 (bases 1 to 1371)
  AUTHORS
  Loetscher, M., Cole, K.E., Strick, C.A., Paradis, T.J., Ogborne, K.T.,
  Gladue, R.P., Lin, W., Boyd, J.G., Moser, B., Wood, D.E.,
  Sahagan, B.G.
  and Neote, K.
  TITLE      Interferon-inducible T cell alpha chemoattractant (I-TAC): a
  novel
  non-ELR CXC chemokine with potent activity on activated T cells
  through selective high affinity binding to CXCR3
  J. Exp. Med. 187 (12), 2009-2021 (1998)
  JOURNAL
  MEDLINE    98290735
  REFERENCE   2 (bases 1 to 1371)
  AUTHORS
  Cole, K.E., Strick, C.A. and Sahagan, B.G.
  TITLE
  JOURNAL
  Submitted (20-OCT-1997) Molecular Sciences, Pfizer, Inc.,
  Eastern
  Point Road, Groton, CT 06340, USA
  FEATURES
  source      Location/Qualifiers
  source      1..1371
  /organism="Homo sapiens"
  /db_xref="taxon:9606"
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  /cell_type="astrocytes"
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  CDS         70..354
  /note="chemokine; I-TAC"
  /codon_start=1

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CDS 102..401
/codon_start=1
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/db_xref="PID:g34519"
/db_xref="SWISS-PROT:P10145"

/translation="MTSKLAVALLAFLISAALCEGAVLPRSAKELRCQCICKTYSKPF

HPKFIKELRVIESGPHCANTEIIVKLSGRELCLDPKENWVQRVVEKFLKRAENS"

mat_peptide 183..398
/note="mat. MDNCF (AA 1 - 72)"

BASE COUNT 526 a 247 c 281 g 506 t

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121 tggctctttt ggcagccttc ctgattttcg cagctctgtg tgaagggtgc gttttgcca
181 ggagtgcata agaactttaga tgtcagtgc taaagacata ctccaaacct ttccacccca
241 aatttatcaa agaactgaga gtgatttggaa gtggaccaca ctgcgccaac acagaaaattt
301 ttgttaaagct ttctgtatggaa agagactctt gtcgtggacc caaggaaaac tgggtgcaga
361 ggggttggaa gaagtttttggaa aagagggtgc agaatttata aaaaaatttca ttctctgtgg
421 tatccaaagaa tcagtgaaga tgccagttaa acttcaagca aatctacttc aacacttcat
481 gtattgtgtg ggtctgttggaa aggggttgc gatgcataac aatgttcgt tggaaatttt
541 aatttcagta aacaatgaat agttttcat tgcatttttttca aatatccaga acataactt
601 atgttaaagta ttatttttttca gaatctacaa aaaacaacaa ataattttta aatataagga
661 ttttcctaga tattgcacgg gagaatatac aaatagccaa atttggccaa gggccaaagag
721 aatattccgaa cttaattttc aggaatttgc tgggtttgc aatatttttca aatatttttca
781 tcacataaaa atgatgggcac aataattttt gccataaaagt ccaatttttgc tggaaatccct
841 ggattttttt ctgttaaatttca tggcaacccct agtctgttag ccaggatccca caagtttttgc
901 ttccactgtg ctttggtttc tcccttattt ctaagtggaa aaagtatttgc ccaccatctt
961 acctcacatgt gatgttgc ggcacatgtgg aagcaacttta agttttttca tcataacata
1021 aatttttttca aatgttacttatttca ttttttttca atgttatttttca ttaagcatca
1081 aatttttttgc caagaatttttgc gaaaataga agatgaatca ttgatttgc ttttttttgc
1141 atgttataatgt aatttttttgc ttttttttgc atttttttgc attttttttgc
1201 atcagggtttt ttagatttttca acaacaaaca atttttttgc cagtttttttgc
1261 atataacaaca aataatttttgc ttttttttgc ttttttttgc
1321 actacaatc ctatgttttgc actcccaatgttgc cagtttttttgc
1381 ttttttttgc ttttttttgc ttttttttgc
1441 ttttttttgc ttttttttgc ttttttttgc
1501 atttttttgc ttttttttgc ttttttttgc

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LOCUS HSINFGER 1172 bp RNA PRI 21-MAR-1995

DEFINITION Human mRNA for gamma-interferon inducible early response gene (with

homology to platelet proteins).

ACCESSION X02530 M17752

NID g33917

KEYWORDS interferon response; signal peptide.

SOURCE human.

ORGANISM Homo sapiens

Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 1172)

AUTHORS Luster, A.D., Unkeless, J.C. and Ravetch, J.V.

TITLE Gamma-interferon transcriptionally regulates an early-response gene

containing homology to platelet proteins

JOURNAL Nature 315 (6021), 672-676 (1985)

MEDLINE 85240552

REFERENCE 2 (bases 1 to 1172)

AUTHORS Luster, A.D.

TITLE Direct Submission

JOURNAL Submitted (29-JUL-1986) to the EMBL/GenBank/DDBJ databases

COMMENT Data kindly reviewed (29-JUL-1986) by Luster A.D.

FEATURES Location/Qualifiers

source 1..1172
/organism="Homo sapiens"
/strain="(U 937 histiocytic lymphoma cell line)"
/db_xref="taxon:9606"

misc_RNA 1
/note="cap site"

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sig_peptide      67..129
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CDS              67..363
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77) "
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                  /db_xref="PID:g33918"
                  /db_xref="SWISS-PROT:P02778"

/translation="MNQTAILICLIFLTLSGIQGVPLSRTVRCTCISISNQPVNPRS

LEKLEIIPASQFCPRVEIIATMKKGKEKRCLNPESKAIKNLLKAVSKEMSKRSP"
mat_peptide      130..360
                  /note="mature early response polypeptide (aa 1-77)"
old_sequence      1138..1141
                  /note="ugaa was uga in [1]"
                  /citation=[1]
old_sequence      1146..1148
                  /note="caa was ca in [1]"
                  /citation=[1]
misc_feature      1155..1160
                  /note="pot. polyA signal"
polyA_site        1172
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BASE COUNT        384 a   231 c   208 g   349 t
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121 attcaaggag tacctcttc tagaaccgta cgctgtaccc gcatcagcat tagtaatcaa
181 cctgttaatc caaggctttt agaaaaactt gaaattattc ctgcaagcca attttgtcca
241 cgttgtgaga tcattgtac aatgaaaaag aagggtgaga agagatgtct gaatccagaa
301 tcgaaggcca tcaagaattt actgaaagca gttagcaagg aaatgtctaa aagatctcct
361 taaaaccaga ggggagcata atcgatgcag tgcttccaaag gatggaccac acagaggctg
421 cctctcccat cacttcccta catggatgtatgcaagcc ataatttttc ttatgttgc
481 gtttactaa aagggtgatca atgttgtca ccaaatcagc tgctactact cctgttaggaa
541 ggttaatgtt catcatccca agctattcag taataactct accctggcac tataatgtaa
601 gctctactga ggtgctatgt tcttagtggta tggcttgacc ctgcttcaaa tattttccctc
661 acctttccca tcttccaagg gtactaaggaa atctttctgc ttggggttt atcagaattt
721 tcagaatctc aaataactaa aagggtgatca atcaaatctc ctttttaaag aatgtctttt
781 acttcatgga ctteccactgc catcctccca aggggcccaa attcttcag tggttaccta
841 catacaattc caaacacata caggaaggta gaaatatctg aaaatgtatg tggtaatgtt
901 cttatataat gaaagactgt acaaagtata agtcttagat gtatatattt cctatattgt
961 tttcagtgtt catgaaataa catgtatc agtactatgt atcaatgagt aacaggaaaa
1021 ttttaaaaat acagatagat atatgtctg catgttacat aagataatg tgctgaatgg
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1141 aagatcaaaa ggttataaa gtaattataa ct
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LOCUS      SYNRPF4A      225 bp      DNA      SYN      15-JUN-1989
DEFINITION Human recombinant platelet factor 4 (PF4) gene, complete cds.
ACCESSION M20901
NID        g209285
KEYWORDS   platelet factor; platelet factor 4.
SOURCE     Synthetic oligonucleotide DNA, clone pIN-III-ompA-2.
ORGANISM   artificial sequence
REFERENCE  1 (bases 1 to 225)
AUTHORS   Barone,A.D., Ghrayeb,J., Hammerling,U., Zucker,M.B. and
          Thorbecke,G.J.
TITLE      The expression in Escherichia coli of recombinant human
platelet   factor 4, a protein with immunoregulatory activity
JOURNAL   J. Biol. Chem. 263, 8710-8715 (1988)
MEDLINE   88243725
FEATURES
  source      Location/Qualifiers
              1..225
              /organism="artificial sequence"
              /db_xref="taxon:29278"
CDS        <1..>225
              /note="recombinant platelet factor 4"
              /codon_start=2

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ACCESSION M36820
 NID g183628
 KEYWORDS cytokine.
 SOURCE Human lymphocyte, cDNA to mRNA, clone GRO-beta.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 1110)
 AUTHORS Haskell,S., Peace,A., Morris,J., Sporn,S.A., Anisowicz,A.,
 Lee,S.W., Smith,T., Martin,G., Ralph,P. and Sager,R.
 TITLE Identification of three related human GRO genes encoding
 cytokine
 functions
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (19), 7732-7736 (1990)
 MEDLINE 91017578
 COMMENT Draft entry and computer-readable sequence for [Proc. Natl.
 Acad.
 Sci. U.S.A. (1990) In press] kindly submitted
 by S.Haskell, 20-JUL-1990.
 FEATURES Location/Qualifiers
 source 1..1110
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 /db_xref="taxon:9606"
 /clone="GRO-beta"
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 gene 75..398
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 CDS 75..398
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 /codon_start=1
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 /db_xref="PID:g183629"

/translation="MARATLSAAPSNPRLRVALLLLLVAASRRAAGAPLATELRCQ
 CLQTLQGIHLKNIQSVKVKSPGPQCAQTEVIATLNGQKACLNPAQMVKKIEKMLK
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BASE COUNT 300 a 247 c 247 g 316 t
 ORIGIN

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    1 gacagagccc gggccacgga gctccttgcg agcttccttc ctgcacacgc cgctcgaaacc
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    241 acatccaaag tggaaatggt aagtcccccgg gaccccaactg cggccaaacc gaactcatag
    301 ccacactcaa gaatggggcag aatggctgtc tcaaccccccgc atgcggatgt gttaaaaaaa
    361 tcatcgaaaaa gatgctgaaa aatggcaaat ccaactgacc agaaggaaagg aggaagctta
    421 ttggtggtcg ttccctgaagg aggcctgccc ttacaggaac agaagaggaa agagagacac
    481 agctgcagag gcccacccggc ttgcgcctaa tgggttttag catactttag agaagtcttc
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    781 actgtgatag aggctgagga atccaaagaaa atggcccgat agatcaatgt gacggcagg
    841 aaatgtatgt gtgtctatatt tggtaactgtt aagatgaatg tcagttgtta ttatttggaa
    901 tgatattcaca gtgtgtgttc aacatttctc atgttgaagc tttaagaact aaaatgttct
    961 aaatatccctt tggcattttt tggatattt gtaagatactt gccttgcgtt atgttattt
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    1081 agaacaggaa aataaaaat taaaaat
  
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LOCUS HUMGROG5 1064 bp mRNA PRI 07-MAR-1995
 DEFINITION Human cytokine (GRO-gamma) mRNA, complete cds.
 ACCESSION M36821
 NID g183632
 KEYWORDS cytokine.
 SOURCE Human lymphocyte, cDNA to mRNA, clone GRO-gamma.
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 REFERENCE 1 (bases 1 to 1064)
 AUTHORS Haskell,S., Peace,A., Morris,J., Sporn,S.A., Anisowicz,A.,

TITLE Lee, S.W., Smith, T., Martin, G., Ralph, P. and Sager, R.
 cytokine Identification of three related human GRO genes encoding
 functions
 JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87 (19), 7732-7736 (1990)
 MEDLINE 91017578
 COMMENT Draft entry and computer-readable sequence for [Proc. Natl.
 Acad. Sci. U.S.A. (1990) In press] kindly submitted
 by S. Haskill, 20-JUL-1990.
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 /clone="GRO-gamma"
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 gene 78..398
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 /db_xref="PID:g183633"

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 BASE COUNT 281 a 237 c 239 g 305 t 2 others
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 841 cttttttttt taaaaaaagaa tggatgtt gtttaggtt ttttttttca aattatctca cattatgtt
 901 tcaacatttt tatgtgttcaag tttcccttag acatttatgt tcttgcttgt agggcataat
 961 gccttggttttaatgtccatc tgccgggtt ctctttccct tggaaaagag aattttatcat
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LOCUS HUMCTAP3 673 bp mRNA PRI 06-MAR-1995
 DEFINITION Human connective tissue activation peptide III mRNA, complete
 cds.
 ACCESSION M54995 M38441
 NID g181175
 KEYWORDS connective tissue activating peptide-III; platelet basic
 protein;
 SOURCE Human platelet, cDNA to mRNA, clone lambda-c[1,2].
 ORGANISM Homo sapiens
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 1 (bases 1 to 673)
 REFERENCE Wenger, R.H., Wicki, A.N., Walz, A., Kieffer, N. and Clemetson, K.J.
 AUTHORS
 TITLE Cloning of cDNA coding for connective tissue activating peptide
 III
 JOURNAL from a human platelet-derived lambda gt11 expression library
 MEDLINE Blood 73 (6), 1498-1503 (1989)
 89229374
 FEATURES Location/Qualifiers
 source 1..673

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/tissue_lib="lambda-gt11"
/map="4p13-q21"
gene 67..453
/gene="PPBP"
sig_peptide 67..168
/gene="PPBP"
/note="G00-127-391"
CDS 67..453
/gene="PPBP"
/codon_start=1
/db_xref="GDB:G00-127-391"
/product="connective tissue activating peptide III"
/db_xref="PID:g181176"



LOCUS HUMENA78A 2177 bp DNA PRI 31-JAN-1996



DEFINITION Homo sapiens neutrophil-activating peptide 78 (ENA-78) gene, complete cds.



ACCESSION L37036 Z46254



NID g607030



KEYWORDS ENA-78 gene; homologue; neutrophil-activating factor; neutrophil-activating peptide 78.



SOURCE Homo sapiens DNA.



ORGANISM Homo sapiens



Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.



REFERENCE 1 (bases 1 to 2177)



AUTHORS Walz,A., Burgener,R., Car,B., Baggolini,M., Kunkel,S.L. and Strieter,R.M.



TITLE Structure and neutrophil-activating properties of a novel inflammatory peptide (ENA-78) with homology to interleukin 8



JOURNAL J. Exp. Med. 174 (6), 1355-1362 (1991)



MEDLINE 92078844



REFERENCE 2 (bases 1 to 2177)



AUTHORS Walz,A.


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TITLE Direct Submission
 JOURNAL Submitted (14-OCT-1994) A. Walz, University of Bern, Theodor
 Kocher
 Institute, Freiestr. 1, Bern, Switzerland 3012
 REFERENCE 3 (bases 1 to 2177)
 AUTHORS Corbett, M.S., Schmitt, I., Riess, O. and Walz, A.
 TITLE Characterization of the gene for human neutrophil-activating
 peptide 78 (ENA-78)
 JOURNAL Biochem. Biophys. Res. Commun. 205 (1), 612-617 (1994)
 MEDLINE 95091791
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ACCESSION  Y08770
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KEYWORDS   cell surface receptor; CXC chemokine; GCP-2 gene; granulocyte
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SOURCE     human.
ORGANISM   Homo sapiens
           Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
           Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE  1 (bases 1 to 254)
           Froyen,G., Proost,P., Ronsse,I., Mitera,T., Haelens,A.,
           Wuyts,A.,
           Opdenakker,G., Van Damme,J. and Billiau,A.
           Cloning, bacterial expression and biological characterization
           of recombinant human granulocyte chemotactic protein-2 and
           differential expression of granulocyte chemotactic protein-2
           and
           epithelial cell-derived neutrophil activating peptide-78 mRNAs
JOURNAL    Eur. J. Biochem. 243 (3), 762-769 (1997)
MEDLINE    97210779
REFERENCE  2 (bases 1 to 254)
           Froyen,G.F.V.
TITLE      Direct Submission
JOURNAL    Submitted (10-OCT-1996) G.F.V. Froyen, Rega Institute,
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DEFINITION Human DNA for SCM-1beta precursor, complete cds.
ACCESSION D63789
NID g1754608
KEYWORDS SCM-1beta; SCM-1beta precursor.
SOURCE Homo sapiens placenta DNA, clone:hg44.
ORGANISM Homo sapiens
Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
REFERENCE 1 (sites)
AUTHORS Yoshida,T., Imai,T., Kakizaki,M., Nishimura,M. and Yoshie,O.
TITLE Molecular cloning of a novel C or gamma type chemokine, SCM-1
JOURNAL FEBS Lett. 360 (2), 155-159 (1995)
MEDLINE 95180438
REFERENCE 2 (sites)
AUTHORS Yoshida,T., Imai,T., Takagi,S., Nishimura,M., Ishikawa,I.,
Yaoi,T.
TITLE Structure and expression of two highly related genes encoding
SCM-1/human lymphotactin
JOURNAL FEBS Lett. 395 (1), 82-88 (1996)
MEDLINE 97002294
REFERENCE 3 (bases 1 to 5669)
AUTHORS Yoshida,T.
JOURNAL Unpublished (1995)
REFERENCE 4 (bases 1 to 5669)
AUTHORS Yoshida,T.
TITLE Direct Submission
JOURNAL Submitted (07-AUG-1995) to the DDBJ/EMBL/GenBank databases.
Tetsuya
Mishima, Yoshida, Shionogi Institute for Medical Science; 2-5-1,
Settsu, Osaka 566, Japan (E-
mail:teyoshid@fl.lab.shionogi.co.jp,
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DEFINITION Human DNA for SCM-1alpha precursor, complete cds.
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Vertebrata; Mammalia; Eutheria; Primates; Catarrhini;
Hominidae; Homo.
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AUTHORS Yoshida,T., Imai,T., Kakizaki,M., Nishimura,M. and Yoshie,O.
TITLE Molecular cloning of a novel C or gamma type chemokine, SCM-1
JOURNAL FEBS Lett. 360 (2), 155-159 (1995)

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 AUTHORS Yoshida,T., Imai,T., Takagi,S., Nishimura,M., Ishikawa,I.,
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 and Yoshie,O.
 TITLE Structure and expression of two highly related genes encoding
 SCM-1/human lymphotactin
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 Tetsuya Yoshida, Shionogi Institute for Medical Science; 2-5-1,
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DEFINITION Human CX3C chemokine precursor, mRNA, alternatively spliced,
complete cds.
ACCESSION U91835
NID g1899258
KEYWORDS
SOURCE human.
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Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1635)
AUTHORS Bazan,J.F., Bacon,K.B., Hardiman,G., Wang,W., Soo,K., Rossi,D.,
Greaves,D.R., Zlotnik,A. and Schall,T.J.
TITLE A new class of membrane-bound chemokine with a CX3C motif
JOURNAL Nature 385 (6617), 640-644 (1997)
MEDLINE 97177111
REFERENCE 2 (bases 1 to 1635)
AUTHORS Bazan,J.F., Bacon,K.B., Hardiman,G., Wang,W., Rossi,D.,
Greaves,D.R., Zlotnik,A. and Schall,T.J.
TITLE Direct Submission
JOURNAL Submitted (03-MAR-1997) Molecular Biology, DNAX Research
Institute, 901 California Ave., Palo Alto, CA 94304-1104, USA
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 SOURCE human.
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 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
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 REFERENCE 1 (bases 1 to 3310)
 AUTHORS Bazan, J.F., Bacon, K.B., Hardiman, G., Wang, W., Soo, K., Rossi, D.,
 Greaves, D.R., Zlotnik, A. and Schall, T.J.
 TITLE A new class of membrane-bound chemokine with a CX3C motif
 JOURNAL Nature 385 (6617), 640-644 (1997)
 MEDLINE 97177111
 REFERENCE 2 (bases 1 to 3310)
 AUTHORS Bazan, J.F., Bacon, K.B., Hardiman, G., Wang, W., Rossi, D.,
 Greaves, D.R., Zlotnik, A. and Schall, T.J.
 TITLE Direct Submission
 JOURNAL Submitted (07-JAN-1997) Molecular Biology, DNAX Research
 Institute, 901 California Ave., Palo Alto, CA 94304-1104, USA
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 REFERENCE 1 (bases 1 to 1430)
 AUTHORS Hedrick,J.A., Helms,A., Gorman,D. and Zlotnik,A.
 TITLE Identification of a novel human CC chemokine upregulated by IL-
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 JOURNAL Blood (1998) In press
 REFERENCE 2 (bases 1 to 1430)
 AUTHORS Hedrick,J.A., Helms,A., Gorman,D. and Zlotnik,A.
 TITLE Direct Submission
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 901 California Ave, Palo Alto, CA 94304, USA
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/26291

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/84, 85.1, 184.1, 186.1, 188.1, 278.1; 514/2, 8, 12, 44; 530/300, 324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,141, 867 A (IVANOFF et al.) 25 August 1992, see entire document.	22-32, 45-55
A	ENG et al. The Stimulatory Effects of Interleukin (IL)-12 On Hematopoiesis Are Antagonized by IL-12-induced Interferon γ In Vivo. J. Exp. Med. May 1995, Vol.181, pages 1893-1898, see entire document.	1-21, 33-44
A	ORANGE et al. Mechanism of Interleukin 12-mediated Toxicities during Experimental Viral Infections: Role of Tumor Necrosis Factor and Glucocorticoids. J. Exp. Med. March 1995, Vol.181, pages 901-914, see entire document.	1-21, 33-44

<input checked="" type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
A	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 25 MARCH 1999	Date of mailing of the international search report 15 APR 1999
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/26291

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WU et al. Receptor-mediated in Vitro Gene Transformation by a Soluble DNA Carrier System. The Journal of Biological Chemistry. 05 April 1987, Vol.252, No. 10, pages 4429-4432, see entire document.	22-32, 45-55

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/26291

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/26291

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07K 14/47, 14/52; C12N 15/12, 15/19, 15/63; A61K 38/16, 38/19, 48/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/84, 85.1, 184.1, 186.1, 188.1, 278.1; 514/2, 8, 12, 44; 530/300, 324

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAN ONLINE, MEDLINE, CAPLUS

search terms: chemokine, vaccination, immunogenic, antigen, HIV, efficacy, macrophage-derived chemokine, stromal cell-derived factor, monocyte chemotactic protein, composition, administration

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-21, 33-44, drawn to a method to enhance the efficacy of a vaccine in a subject comprising administering an antigen and one or more chemokines and a composition thereto.

Group II, claims 22-32, 45-55, drawn to a method to enhance the efficacy of a vaccine in a subject comprising administering nucleic acid sequences encoding one or more antigens and nucleic acid sequences encoding one or more chemokines.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product and method, a method to enhance the efficacy of a vaccine in a subject comprising administering an antigen and one or more chemokines and a composition thereto. Further pursuant to 37

C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.